AD-A240 058

MENTATION PAGE

Form Approved
OMB No. 0704-0188

	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	23 Jul 91	FINAL 01 Jul 87 to 30 Apr 91

4. TITLE AND SUBTITLE
Validation and Application of Pharmacokinetic Models for Interspecies Extrapolations in Toxicity Risk Assessments of Volatile Organics

5. FUNDING NUMBERS
GR - AFOSR-87-0248
PE - 61102F
PR - 2312
TA - A5

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8. PERFORMING ORGANIZATION REPORT NUMBER
AFOSR-TR- 91 0735

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Lt Col Cerveny
Air Force Office of Scientific Research
Building 410
Bolling AFB DC 20332-6448

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words) Direct measurements of perchloroethylene (PER), trichloroethylene (TCE), trichloroethane (TRI) and dichloroethylene (DCE) were made in the blood and exhaled breath of rats during and following inhalation exposures. The pharmacokinetics of these four halocarbons were also investigated following oral administration. An accurate assay for measuring volatile halogenated hydrocarbons in a variety of body tissues was developed and demonstrated for PER, TCE, TRI, and tetrachloroethane (TET). The tissue concentration-time profiles and bioavailability for PER and TET were determined in liver, kidney, brain, fat, lung, heart, and muscle tissues following oral and intraarterial administrations in rats. Interspecies comparisons of the pharmacokinetics of PER and TET were made following oral and intraarterial administrations in two species: Sprague-Dawley rats and Beagle dogs. Neurobehavioral determinations were conducted in rats following inhalation exposures, single oral bolus administration, and gastric infusion of PER. A physiologically-based pharmacokinetic (PBPK) computer simulation model was developed and validated for the accurate prediction of PER, TRI, TET and TCE pharmacokinetics using the direct measurements data, thus demonstrating a robust model which could simulate the uptake, disposition, and elimination of halocarbons with a wide variation in physicochemical properties.

14. SUBJECT TERMS			15. NUMBER OF PAGES / 16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(UNCLASSIFIED)	(UN CLASSIFIED)	(UNCLASSIFIED)	UNLIMITED

FINAL TECHNICAL REPORT

Validation and Application of Pharmacokinetic Models for Interspecies Extrapolations in Toxicity Risk Assessments of Volatile Organics

Grant # AFOSR 870248
Life Sciences Directorate
Air Force Office of Scientific Research

Dr. Cham E. Dallas, Principal Investigator

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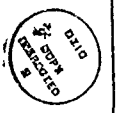

AFOSR-TR- 91 0735

VALIDATION AND APPLICATION OF PHARMACOKINETIC MODELS FOR INTERSPECIES EXTRAPOLATIONS IN TOXICITY RISK ASSESSMENTS OF VOLATILE ORGANICS

Approved for public release;
distribution unlimited.

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Final Report for Period 1 July 1987 - 30 April 1991

Prepared for

Life Sciences Directorate
Air Force Office of Scientific Research
Bolling Air Force Base, DC 20332-6448

APPENDICES

I. OVERALL OBJECTIVE AND STATEMENT OF WORK

The overall objective of the proposed project is to investigate the scientific basis for interspecies extrapolation of pharmacokinetic and neurobehavioral toxicity data. Direct measurements of blood and tissue concentrations of halocarbons over time in two species will be used to formulate and validate physiologically-based pharmacokinetic models for inhalation and oral exposure. These models will be used for: (a) prediction of the time-course of blood and target organ levels in the absence of data; (b) interspecies extrapolations (i.e. scale-up from smaller to larger laboratory animals and ultimately to man). A combined physiological pharmacokinetic-toxicodynamic model for inhalation exposure to halocarbons will also be developed and evaluated for its ability to predict neurobehavioral effects under specified exposure conditions.

A series of experiments will be conducted to provide a pharmacokinetic data base of interspecies comparisons and for formulation of physiologically-based pharmacokinetic models. Adult male Sprague-Dawley rats and male beagle dogs will be administered equal doses/concentrations of selected halocarbons. Ingestion, inhalation and intravenous injection will be employed as routes of administration. Concentrations of the parent compounds will be monitored in the blood (and in some cases in the exhaled breath) for appropriate periods during and after exposures. The cumulative uptake from exposure to each chemical will be determined. Relative rates and magnitude of elimination of the test chemicals by metabolism and respiration will be evaluated. For investigating the relative role of metabolism on the observed pharmacokinetics of volatile organic compounds, trichloroethylene (TCE), dichloroethylene (DCE), and trichloroethane (TRI) will be employed. Tetrachloroethylene (PER) and 1,1,2,2-tetrachloroethane (TET) will be employed for analyzing the role of pulmonary extraction/elimination. At least 2 doses and 2 vapor concentrations of each pair of test compounds will be utilized.

In order to determine the tissue disposition of halocarbons in two species, rats and dogs will receive equivalent exposures to halocarbons intravenously, orally and by inhalation. Concentrations of the parent compound in brain, liver, kidney, heart, lung, skeletal muscle and adipose tissue will be measured at selected intervals over time, in order to provide an assessment of the actual target organ dose for validation of physiologically-based model development and inter-species correlations with toxicity. A second series of tissue disposition experiments will be conducted to determine what adjustments in administered dose are necessary to achieve equal brain levels of test compounds in each species. An inhalation and oral exposure concentration will be administered to the rat that yields a brain level of halocarbon similar to that seen in the previous experiments in the dog.

Physiologically-based pharmacokinetic models will be developed and validated for oral and inhalation exposures to halocarbons, which will allow accurate prediction of the concentration of halocarbons in blood and tissues over time during and following exposure. Data from the direct measurements of blood and tissue concentrations will be compared to simulated values calculated from mass-balance differential equations comprising the model. Thereby, the accuracy of the model can be tested and adjustments made where necessary to improve the

model simulations. Models for inhalation and oral exposures validated using pharmacokinetic data in rats will be employed to predict blood and tissue halocarbon concentrations in the dog. The accuracy of the model for this interspecies extrapolation will be assessed by comparing the predicted concentrations to values determined experimentally for the dog.

The target organ concentration will be correlated with the neurobehavioral toxicity of inhaled solvents. The magnitude of CNS effects of each solvent will be correlated with the target organ (i.e. brain) concentration, as determined in the tissue uptake studies, at each time-point. In this manner, the feasibility of toxicity extrapolations between species based on a common target organ dose will be evaluated. Toxicodynamic models will then be developed and validated for inhaled halocarbons. To develop this model, rat brain halocarbon concentrations will be correlated with the magnitude of neurobehavioral effects in an appropriate equation and the validity of the equation tested by comparison with actual experimental values obtained from previous stages of the project. By incorporating predicted brain concentrations from the previously validated physiologically-based pharmacokinetic model, a combined physiological pharmacokinetic-toxicodynamic model can be developed. This combined model may allow the prediction of toxicity from the interspecies extrapolation of pharmacokinetic data, and in simulations in the absence of experimental data.

II. INVESTIGATION OF THE RELATIVE ROLE OF METABOLISM IN THE PHARMACOKINETICS OF INHALED HALOCARBONS.

One of the major objectives was to fully characterize the pharmacokinetics of inhaled halocarbons during and following exposures, in order to provide a pharmacokinetic data base for interspecies comparisons and for formulation of physiologically-based pharmacokinetic models. These studies have been conducted with the Sprague-Dawley rat. These comprehensive experiments will provide the information necessary for the most efficacious design of experimental protocols for the dog. This will aid in avoiding inefficient or redundant work in the dog experiments, an important factor in view of the high cost of conducting experiments in dogs. Concentrations of the parent compounds were monitored in the blood and in the exhaled breath for appropriate periods during and after exposures in order to delineate uptake and elimination of the test chemicals. These data were then subjected to pharmacokinetic analyses and subsequently used in formulation of physiologically-based computer simulation models.

A major route of elimination of halocarbons is hepatic metabolism. If metabolism plays a significant role in the disposition and subsequent neurobehavioral effects of these chemicals, extensively metabolized halocarbons should be more rapidly eliminated (and have a less pronounced CNS depressant action, as determined in subsequent experiments than poorly metabolized halocarbons. In order to test this PREMISE, halocarbons with widely differing propensity for metabolism were studied. Extensively metabolized trichloroethylene (TCE) and dichloroethylene (DCE) and poorly metabolized 1,1,1-trichloroethane (TRI) were used. DCE and TRI are of comparable volatility, so they would be expected to be eliminated similarly by the lung. Differences in pharmacokinetics could therefore more likely be attributed to differences in

metabolism. While TCE and TRI are very similar structurally (differing only in a single double bond), differences in both volatility and metabolism should be reflected in the resulting uptake and elimination of the test chemicals.

For these inhalation exposures, the halocarbon was administered to unanesthetized male Sprague-Dawley rats previously prepared with an indwelling carotid artery cannula. These rats, weighing 325-375 g, inhaled the compound for 2 hr through a one-way breathing valve in an inhalation exposure system previously developed by this laboratory. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently and analyzed for the test compound. Respiratory rates and volumes were continuously monitored during and following exposure, and were used in conjunction with the pharmacokinetic data to characterize profiles of uptake and elimination. This experimental protocol has provided a unique approach by combining direct measurements of the halocarbons TCE, TRI, and DCE in the exhaled breath and blood simultaneously with detailed measurements of respiration. The separation of the inhaled and exhaled breath streams by use of the one-way breathing valve afforded both sampling of the exhaled breath of halocarbon during and following exposure and measurement of the air flow in the breath stream. The breathing valve has been used previously for monitoring respiration in unanesthetized animals (Mauderly et al., 1979), but pharmacokinetic measurements were not made using this system. In previously reported pharmacokinetic studies of inhaled halocarbon in laboratory animals, direct determinations of the exhalation of the solvent by individual animals during exposures were not made, as most of these studies employed dynamic or closed exposure chambers. Emphasis on the pharmacokinetic measurements of these halocarbons has focused primarily on measurements following the termination of exposure. Also, parameters of respiration were not monitored in these experiments. Accurate determination of the total amount of chemical absorbed or eliminated by inhalation requires monitoring of respiratory parameters. In the present study, measurement of halocarbon uptake was accomplished by calculation from either the blood level data or the exhaled breath data in conjunction with the monitored respiratory parameters.

III. STUDIES OF THE PHARMACOKINETICS OF INHALED TRI IN RATS

Studies of the pharmacokinetics of TRI in rats during and following inhalation exposure, including the validation of a PBPK model for TRI pharmacokinetics, have been completed. The results have been published in a respected peer-reviewed scientific journal. The reprint is included as Section A of the Appendix (and listed in Appendix M), and the reference is as follows:

> Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "The uptake and elimination of 1,1,1-trichloroethane (TRI) during and following inhalation exposure in rats. Toxicology and Applied Pharmacology 68: 140-151 (1989).

It was found that TRI was very rapidly absorbed from the lung, in that substantial levels were present in arterial blood at the first sampling time, (i.e., 2 min). TRI blood and exhaled breath levels increased rapidly after the initiation of exposure to near steady-state within approximately 20-45 min and were then directly proportional to the exposure concentration. Percent uptake

decreased over time during inhalation exposures until equilibrium was established by 1 hr, after which % uptake of the inhaled dose was approximately 50% for both exposure groups. Total cumulative uptake of 50 and 500 ppm TRI over the 2-hr inhalation exposures was determined to be 6 and 48 mg/kg bw, respectively. By the end of the exposure period, 52.5 and 56.3% of the total inhaled dose was eliminated in the breath of the low and high dose groups, respectively. A physiological pharmacokinetic model for TRI inhalation was utilized to predict blood and exhaled breath concentrations for comparison to observed experimental values. Overall, values predicted by the physiological pharmacokinetic model for TRI levels in the blood and exhaled breath were in close agreement with measured values both during and following TRI inhalation. While TRI exhaled breath levels in rats in this study were comparable to those measured previously in humans, blood levels of TRI were not equivalent in rats and in man. It was therefore concluded that the rat may be a potential useful model for TRI respiratory elimination in man.

IV. STUDIES OF THE PHARMACOKINETICS OF TCE FROM INHALATION EXPOSURE IN RATS

A second paper on the pharmacokinetics and PBPK model validation for inhaled halocarbons has been completed, and it describes the uptake and disposition of trichloroethylene (TCE) in rats during the following inhalation exposure. This paper is currently in press at a peer-reviewed scientific journal. The galley proof is included as Section B of the Appendix (and listed in Appendix M), and the reference is as follows:

> Dallas, C.E., Gallo, J.M., Ramanathan, R., Muralidhara, S., and Bruckner, J.V. "Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats." In press, Toxicology and Applied Pharmacology (1991).

In this study, the effect of the saturation of the metabolism of TCE during inhalation exposures on the subsequent pharmacokinetics of the compound was evaluated. TCE exhaled breath levels were found to have increased rapidly after the initiation of exposure to near steady-state within approximately 20-30 min and were then directly proportional to the exposure concentration. Uptake of TCE in the blood was also rapid, but blood levels continued to increase progressively over the course of the inhalation exposure at both dose levels. Arterial TCE concentrations were not proportional to the inhalation concentration, with levels for the 500 ppm group from 25-30 times greater than in 50 ppm-exposed rats during the second hour of the exposure. Percent uptake was nearly complete at the initiation of inhalation exposure and decreased rapidly thereafter until equilibrium was established by 1 hr, after which % uptake of the inhaled dose was approximately 69-72% for both exposure groups. Total cumulative uptake of 50 to 500 ppm TRI over the 2-hr inhalation exposures was determined to be 8.4 and 73.3 mg/kg bw, respectively. The direct measurements of TCE in the blood and exhaled breath were utilized in the validation of a physiological pharmacokinetic model of the prediction of the pharmacokinetics of inhaled TCE. Results from this study indicate that metabolism of TCE is saturable between 50 and 500 ppm exposure in rats, resulting in disproportionately higher blood levels above the saturation point. At doses below this metabolism saturation point in rats, blood and exhaled breath levels

of TCE in rats were very similar to values previously published for TCE inhalation exposures in humans.

V. STUDIES OF THE PHARMACOKINETICS OF INHALED DCE IN RATS

An investigation of the uptake, disposition, and elimination of 1,1-dichloroethylene (DCE) has also been completed for inhalation and oral exposures in rats. These results were presented at the most recent meeting of the Society of Toxicology in March, 1988. The reference for these studies (also listed in Appendix M) as presented in the abstract is as follows:

> Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "Comparative pharmacokinetics of inhaled and ingested 1,1-dichloroethylene (DCE) in rats." 27th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 8: 139 (1988).

A manuscript from this data with the same authors and title has been prepared for submission to a peer-reviewed scientific journal. It is included in the Appendix as Section G, and the reference for the manuscript (also listed in Appendix M) is:

> Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V., "Comparative pharmacokinetics of inhaled and ingested 1,1-dichloroethylene in rats." (to be submitted to Toxicology and Applied Pharmacology, 1991).

In this study, the uptake, disposition, and elimination of DCE were determined during and following 100 and 300 ppm inhalation exposures for 2 hours. Oral exposures to DCE were investigated utilizing 10 and 30 mg/kg doses administered as either a) a single oral bolus; b) a gastric infusion for 2 hours; or c) three equally divided bolus doses over a 2 hour period. Significant respiratory elimination of unchanged DCE was evident during the inhalation exposure period, with steady-state DCE levels achieved in the exhaled breath within 20 min at both dose levels. DCE respiratory elimination was proportional to the inhaled concentration during exposure. As a result of the 2-hr exposure to 100 ppm DCE the cumulative uptake was 3.3 $\pm$ 0.3 mg ($\bar{x} \pm$ SE), or 10 mg/kg bw. The total cumulative uptake of DCE from the 2-hr exposure to 300 ppm was 10.2 $\pm$ 0.6 mg ($\bar{X} \pm$ SE), or 30 mg/kg bw. Percent uptake of DCE during inhalation exposure was similar in magnitude at both exposure concentrations. The magnitude of pulmonary elimination was proportional to the inhalation exposure concentration.

Arterial DCE concentrations, however, were not proportional to the inhalation concentration. After the initial rapid uptake phase over the first 20 minutes of exposure, blood levels for the 300 ppm-exposed rats were 4 to 5 times higher than DCE blood concentrations of rats that received 100 ppm exposures. The maximum blood levels achieved during single oral bolus or gastric infusion administration were also not proportional to the administered dose. As with the inhalation exposures, these blood values for the high dose group were at least 4 to 5 times higher than for the low dose group of both oral administrative routes. The Cmax achieved for the multiple bolus administration

of 30 mg/kg, however, was nearly 9 times more than that achieved following 10 mg/kg.

The AUC values for the single bolus and gastric infusion groups were similar at both dose levels (nearly identical for the high dose). These oral administration values were only 60-80% of the corresponding inhalation AUCs. The bioavailability (F) of DCE was determined by the ratio of the AUC value of each experimental group to the corresponding dose administered by intravenous administration. The high dose groups consistently had a higher F than the groups administered the low dose of DCE by any of the exposure routes. At both the high and low doses, F was higher for animals inhaling DCE than for orally administered rats.

VI. STUDIES OF THE PHARMACOKINETICS OF INGESTED TRI, TCE, AND DCE

A variety of halocarbons and other VOCs have been identified as contaminants of food and drinking water supplies in the U.S. (Symons et al., 1975; NOMS, 1977). Some of the halocarbons most commonly identified in water supplies are 1,1-dichloroethylene (1,1-DCE), trichloroethylene (TCE), and 1,1,1-trichloroethane (TRI). As indicated previously, in addition to being important as environmental contaminants these agents have been selected for investigation in order to evaluate the relative role of the saturation of metabolism of the compounds after exposure. Emissions from product manufacturing, usage activities, and spills are though to be primary sources of these halocarbons in water supplies. Recently, the contamination of drinking water supplies by the leakage of solvents from storage tanks and chemical waste dumps has become of significant concern. There are large numbers of solvent and fuel storage tanks in the nation, many at U.S. Air Force facilities. As the majority of these are located underground, leakage of solvents into groundwater supplies can proceed undetected for years.

Despite the potential public health significance of halocarbon ingestion from contaminated drinking water supplies, there is presently insufficient information available concerning the systemic absorption and disposition of these and other halocarbons following their oral administration. Most studies have involved administration of ^{14}C-labeled halocarbons and measurement of levels of radioactivity at a single time-point following dosing, thought (his approach precludes delineating between parent compound and metabolites. While blood and tissue levels of halocarbons have usually been measured only 2 or 3 days after oral dosing, ingestion of certain halocarbons has been shown to result in pronounced cytotoxic effects within minutes or hours of ingestion (Moore et al., 1976; Lowrey et al., 1981; Luthra et al., 1984). Thus it is important to know the extent of systemic absorption and disposition of halocarbons in the body during the period immediately following ingestion. Therefore, investigations toward this end were initiated by this laboratory during a previous grant effort, resulting in preliminary data on ingested DCE (Putcha et al., 1986) and TCE (D'Souza et al., 1985). These studies were conducted in rats anesthetized with ether, with the compound administered with polyethylene glycol as a dosage vehicle. A major goal of these studies was to compare pharmacokinetics of the compounds in fed and fasted animals, in which it was found that food appears to delay the absorption of the halocarbons from the gut. The bioavailability of these agents was equivalent in animals given the same dose by oral or intravenous

administration. Therefore, it is apparent that absorption of the orally administered halocarbon is complete.

In the present investigation, the halocarbon used as test chemicals were administered to unanesthetized rats. The disadvantages of using anesthesia during chemical exposures were thus avoided. Anesthetics such as phenobarbital and dimethyl ether are known to inhibit the metabolism of drugs which are biotransformed by the hepatic mixed function oxidase system (Johanssen et al., 1981; Vermeulen et al., 1983). It is possible that use of anesthesia may alter the metabolism and kinetics of halocarbons during exposure. Accordingly, we utilized an unanesthetized animal model approach in order to be certain that the halocarbon pharmacokinetic results obtained were representative of kinetics in unanesthetized, relatively unstressed animals.

As anesthetics have a potential to affect respiration rates and volumes, they could alter the quantity of test chemical which is eliminated. For evaluating the oral pharmacokinetics of chemicals like halocarbons, this can be a very important factor since halocarbon elimination in the breath has been shown to be a significant factor following halocarbon ingestion (Chieco et al., 1981; Dallas et al., 1986). Increased or decreased respiration may alter the rate and magnitude of the respiratory excretion of the halocarbon, thus affecting systemic kinetics as well. Other problems such as anesthetic-induced fluctuations in body temperature would also be avoided by use of an unanesthetized animal model. Significant changes in body temperature have an impact on enzyme systems that can alter the metabolism and pharmacokinetics of certain chemicals. While core temperature can be monitored and periodically readjusted with a heating pad for anesthetized animals, some changes are inevitable with the system. Also, possible competitive metabolic inhibition and alteration in transport processes by anesthetics are avoided in an unanesthetized model.

Studies of the dose dependence of the pharmacokinetics of ingested DCE in unanesthetized rats have been completed. These results were presented as part of the Society of Toxicology abstract previously cited on page 5. Previous studies of DCE in rats have indicated that lethality (Andersen and Jenkins, 1977) and hepatotoxicity (Andersen et al., 1979a) have abrupt increases in response over a definite range of DCE exposure concentration. Saturation of the metabolic activation of DCE is believed to occur due to depletion of glutathione, leading to this sudden increase in toxicity. This capacity of the rat to metabolize DCE may have been exceeded by a single oral dose between the range of 50 to 100 mg/kg (Andersen and Jenkins, 1977; McKenna et al., 1978). In the studies thus far completed, the pharmacokinetics of DCE in unanesthetized rats have been evaluated over a range of doses below this perceived metabolic saturation point for DCE ingestion, with additional dose studies expected to exceed DCE metabolism in the rat to be conducted later.

In order to procure repetitive blood samples following administration of single oral bolus doses of halocarbons to unanesthetized rats, an indwelling arterial cannula was surgically implanted prior to the halocarbon exposure. The cannula was tunneled subcutaneously to the back of the animal and exited just behind the head. The cannula was extruded through a steel spring that was attached to the back of the animal by a harness. After the surgery was complete, the animal was placed into a metabolism cage to recover for 24 hours before

halocarbon dosing. The steel spring was exited through, the top of the cage and connected to a counter-balance weight system, which prevented the animal from interfering with the cannula but allowed relative freedom of movement.

The rats were given the halocarbon in an aqueous Emulphor emulsion as a single oral bolus dose of 10 and 30 mg/kg DCE. Blood samples were taken from the carotid artery cannula for up to 5 hours following the oral dose. The halocarbon content of the blood samples was measured with a gas chromatograph equipped with a semi-automatic headspace sampler and an electron capture detector.

The total body clearance (CL_T) of 1,1-DCE was calculated using the formula:

$$CL_T = \frac{Dose}{\int_0^{\infty} Cdt}$$

where ${}_0\int^{\infty}Cdt$ is the are under the blood concentration versus time curve (AUC) of 1,1-DCE.

Apparent volume of distribution (V_β) was calculated using the formula:

$$V_\beta = \frac{Dose}{\beta_0\int^{\infty} Cdt}$$

The biological half-life ($t_{\frac{1}{2}}$) of 1,1-DCE was calculated by the formula:

$$t_{\frac{1}{2}} = \frac{0.693}{\beta}$$

where ß is the terminal elimination rate constant.

The volume of distribution of the central compartment (V_c) was calculated using the formula:

$$V_c = \frac{Dose}{P+A+B}$$

where P, A, and B are the 0-time intercepts of the three exponential phases of the blood concentration versus time curves.

The blood concentration-time profiles for the oral administration of DCE in unanesthetized rats are shown in Fig. D-1. DCE was very rapidly absorbed from the gut, as peak blood levels of DCE were reached within 4 minutes after oral bolus dosing. Comparison of the pharmacokinetic parameters between the low dose groups are shown in Table D-1. The maximum blood concentration (C-MAX) reached following 30 mg/kg dosing was 4.3 times that achieved following oral administration of 10 mg/kg. Comparing the area-under-the-blood-concentration-time curve (AUC) also indicated a disproportionate difference of 4.7 times between the two dose groups. The elimination half-lives ($t_{\frac{1}{2}}$) were similar at the two dose levels, however. Both the apparent clearance and volume of distribution

of the 30 mg/kg dose group were approximately two-thirds that of the 10 mg/kg group. Compared to the previous data from the oral administration of DCE in anesthetized rats by this laboratory (Putcha et al., 1986), the terminal elimination $t_{\frac{1}{2}}$ and AUC were lower in the current investigation with unanesthetized rats. Using a 10 mg/kg dose in both studies as an example, the mean $t_{\frac{1}{2}}$ and AUC were lower in the current investigation with unanesthetized rats. Using a 10 mg/kg dose in both studies as an example, the mean $t_{\frac{1}{2}}$ and AUC values for the anesthetized rats were 181 μg-min/ml and 78.2 minutes, respectively, while these two parameters for unanesthetized rats were 50.5 μg-min/ml and 50 minutes, respectively. These differences, however, might not be attributable only to the use of anesthesia. The previous study with anesthetized rats also employed 50% aqueous polyethylene glycol (PEG) 400 as a diluent, while an aqueous emulsion (emulphor) was used in the present investigation.

The investigation of the pharmacokinetics of TRI following oral administration as a single bolus in unanesthetized rats has also been completed. These results were presented at the most recent meeting of the Society of Toxicology in Dallas, Texas. It is intended that this study will be combined with the results of experiments currently underway involving the intravenous administration of TRI for the submission of a manuscript to a peer-reviewed journal by the end of the year. The reference for the study of orally-administered TRI (also listed in Section M of the Appendix) as presented in the abstract is as follows:

> Muralidhara, S., Ramanathan, R., Gallo, J.M., Dallas, C.E., and Bruckner, J.V. "Pharmacokinetics of volatile halocarbons: Comparison of single oral bolus versus gastric infusion of 1,1,1-trichloroethane (TRI)." 27th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 8: 95 (1988).

The experimental protocol for this pharmacokinetic study of TRI was similar to that conducted for DCE. Due to the longer half-life of TRI relative to DCE, though, sampling for ingested TRI was conducted for longer intervals of time (up to 480 minutes after a single oral bolus administration).

Measurements of the systemic uptake, disposition, and elimination of TRI after ingestion in the unanesthetized rat is shown in Fig. D-2. TRI was administered as a single oral bolus dose of either 6 or 48 mg/kg, and samples of arterial blood taken periodically from an indwelling carotid artery cannula and analyzed for TRI by gas chromatography. Samples were taken frequently in the first minutes following the oral dose in order to characterize the very rapid uptake of ingested TRI into the systemic circulation. Arterial blood levels reached a peak 8 to 12 minutes after oral dosing and declined relatively quickly thereafter. The pharmacokinetic parameters for the two dose groups are shown in Table D-2. There was a distinct linear relationship between the maximum blood concentration (C-MAX) achieved from the two doses employed. The AUC values were also approximately proportionate to the administered dose. As was seen for DCE previously, the elimination half-life of TRI was nearly identical for both dose groups (112-115 minutes). Even though DCE and TRI have a similar characteristic volatility (blood:air partition coefficient near 5), the disappearance of ingested DCE from the blood occurs more than twice as fast as does the

disappearance of ingested TRI. This can be attributed to the high rate of metabolism of DCE relative to poorly metabolized TRI.

Studies of the pharmacokinetics of ingested TCE have also been conducted. Results from these experiments were also presented at the most recent meeting of the Society of Toxicology earlier this year. The reference for this study (also listed in Section M of the Appendix) as presented in the abstract is as follows:

> Ramanathan, R., Muralidhara, S., Gallo, J.M., Dallas, C.E., and Brückner, J.V. "Pharmacokinetics of volatile halocarbons: Comparison of single oral bolus versus infusion of trichloroethylene (TCE)." 27th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 8: 94 (1988).

The same procedure was employed for TCE determination after oral exposure as were used previously for DCE and TRI. A single oral bolus of 8 and 76 mg/kg TCE was administered to unanesthetized rats with an indwelling carotid arterial cannula.

The TCE blood concentration-time profile following oral administration is shown in Figure D-3. As was previously observed with DCE and TRI, blood levels of TCE rose quickly after oral dosing to indicate rapid absorption from the gastrointestinal tract. Peak arterial blood levels were reached 8-12 minutes after the single oral bolus dose. The pharmacokinetic parameters for the oral administration of TCE are shown in Table D-3. The AUC for the 76 mg/kg group was 22 times that seen after 8 mg/kg. Due to the known capacity for TCE to saturate the metabolism of the rat with a sufficient dose, this is an indicator that TCE metabolism is possibly saturated by a single oral bolus dose between 8 and 76 mg/kg. Unlike DCE and TRI, which each had similar elimination half-lives ($t_{\frac{1}{2}}$) for dose levels of 3 and 8 fold-difference in magnitude, respectively, the $t_{\frac{1}{2}}$ of TCE was significantly different for the two dose groups. The 76 mg/kg group demonstrated as approximate 50% increase in $t_{\frac{1}{2}}$ relative to the 8 mg/kg group.

VII. EVALUATION OF THE RELATIVE ROLE OF PROPENSITY FOR RESPIRATORY ELIMINATION ON THE PHARMACOKINETICS OF INHALED HALOCARBONS

In the initial phase of this research effort, a series of studies were conducted to evaluate the relative role of hepatic metabolism on the subsequent pharmacokinetics of inhaled halocarbons. Extensively metabolized trichloroethylene (TCE) and dichloroethylene (DCE) and poorly metabolized 1,1,1-trichloroethane (TRI) were used. While TCE and TRI are very similar structurally (differing only in a single double bond), differences in both volatility and metabolism were reflected in the resulting uptake and elimination of the test chemicals. It was important to eliminate the propensity for volatility as a factor in this focus on the impact of metabolism. DCE and TRI are of comparable volatility so they were expected to be eliminated similarly by the lung. The reported differences in pharmacokinetics were therefore more likely to be attributed to differences in metabolism. In this next phase of the research effort, this factor of the propensity for respiratory elimination (due to the characteristic volatility of specific halocarbons) was investigated.

As a class of chemicals, halocarbons have low solubility in blood and high volatility (i.e. low blood:air partition coefficients), as well as rapid

vascular-alveolar transfer. Thus, a substantial proportion of the blood's burden of halocarbons should be removed at each pass through the lungs. It follows that halocarbons with low blood:air partition coefficients should be more efficiently eliminated (and have a less prolonged CNS depressant action) than halocarbons with relatively high partition coefficients. In order to test this premise, tetrachloroethylene (PCE) and 1,1,2,2-tetrachloroethane (TET) will be utilized. Both are poorly metabolized (Ikeda and Ohtsuji, 1972) and have similar oil:blood (i.e. fat/blood) partition coefficients, but PCE has a much lower blood:air partition coefficient (Sato and Nakajima, 1979). In this way, the factors of lipophilicity and propensity for hepatic metabolism are accounted for. Therefore, differences in the pharmacokinetics between PCE and TET in this experimental design are more likely to be attributed to differences in respiratory elimination.

For these inhalation exposures, the halocarbon was administered to unanesthetized male Sprague-Dawley rats previously prepared with an indwelling carotid artery cannula. These rats, weighing 325-375 g, inhaled the compound for 2 hr through a one-way breathing valve in an inhalation exposure system previously developed by this laboratory (see schematic for the inhalation exposure system in Fig. E-1). Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently and analyzed for the test compound. Respiratory rates and volumes were continuously monitored during and following exposure, and were used in conjunction with the pharmacokinetic data to characterize profiles of uptake and elimination. This experimental protocol has provided a unique approach in this present study by combining direct measurements of the halocarbons PCE, TCE, TRI, and DCE in the exhaled breath and blood simultaneously with detailed measurements of respiration. The separation of the inhaled and exhaled breath streams by use of the one-way breathing valve afforded both sampling of the exhaled breath for halocarbon during and following exposure and measurement of the air flow in the breath stream. Emphasis on the pharmacokinetic measurements of these halocarbons in previous studies has focused primarily on measurements following the termination of exposure. Also, parameters of respiration were not monitored in these experiments. Accurate determination of the total amount of chemical absorbed or eliminated by inhalation requires monitoring of respiratory parameters. In the present study, measurement of halocarbon uptake was accomplished by calculation from either the blood level data or the exhaled breath data in conjunction with the monitored respiratory parameters.

VIII. STUDIES OF THE PHARMACOKINETICS OF PCE DURING AND FOLLOWING INHALATION EXPOSURE IN RATS

Toward this goal of investigating the relative role of the characteristic volatility of a halocarbon on its pharmacokinetics, the uptake, disposition, and elimination of perchloroethylene (tetrachloroethylene, or PCE) has been completed in the rat. The results of this study, together with the PBPK model simulations for inhaled PCE (described in Section XV), were presented at the 28th annual meeting of the Society of Toxicology in Atlanta, Georgia in March, 1989. A manuscript from this work is also in preparation for submission to a peer-reviewed journal this fall. The reference for this study (also listed in Appendix M) as presented in the abstract is as follows:

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., Manning, R.O., and Bruckner, J.V. "Direct measurements of perchloroethylene in the blood and exhaled breath of rats during and following inhalation exposure." 28th Annual Meeting of the Society of Toxicology, Atlanta, GA; Toxicologist 9: 78 (1989).

Specific objectives of this study were to: 1) provide accurate direct measurements of the respiratory uptake and elimination of PCE during and following inhalation exposures by simultaneously measuring PCE in the blood and exhaled breath; 2) determine the total dose of PCE absorbed systematically (cumulative uptake) during 2-hour inhalation exposures using inhaled and exhaled breath determinations and the monitored volumes of respiration; 3) assess the effect of a 10-fold difference in exposure concentration (50 and 500 ppm) on PCE uptake and elimination from the blood, total cumulative uptake, and elimination in the breath; 4) validate a physiologically-based pharmacokinetic (PBPK) model for PCE inhalation by comparing computer simulations of PCE uptake and elimination with experimentally observed values. Fifty or 500 ppm PCE was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 325-375 g (as described in detail in Appendix A). Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected during and following per inhalation and analyzed by gas chromatography.

In order to calculate the total received dose of PCE during inhalation exposures, the respiration of each animal was continuously monitored. The respiratory monitoring technique was conducted according to the methods previously published in solvent exposure studies by this laboratory (Dallas et al., 1983, 1986 and 1989). The airflow created by the animal's inspiration was recording both during and following PCE inhalation exposure in terms of minute volume (volume of respiration per minute, V_E), respiratory rate (f), and tidal volume (V_T). An average value for these parameters for each individual animal was obtained by averaging the measurements taken at 15-min intervals during the 2-hr exposure. The mean $\pm$ SD of these average values for the 500 ppm exposure group (n=6) were: V_E = 189 $\pm$ 21.5; f = 119.1 $\pm$ 22.4; V_T = 1.62 $\pm$ 0.34. The mean $\pm$ SD for these average values for the 50 ppm exposure group (n=6) were: V_E = 216 $\pm$ 43.1; f = 134.5 $\pm$ 14.9; V_T = 1.67 $\pm$ 0.36.

Significant respiratory elimination of unchanged PCE was evident during the inhalation exposure period, with near steady-state PCE levels achieved in the exhaled breath within 20-30 min. These near-steady state concentrations were approximately 2.1-2.4 μg/ml in the exhaled breath of the 500 ppm exposed rats (Fig. 2, Appendix E, or Fig. E-2). In the 50 ppm inhalation exposure group, these exhaled breath levels at near-steady state were in the range of 0.20-0.22 μg/ml (Fig. E-3). PCE was readily absorbed from the lung, in that substantial levels of PCE were present in the arterial blood at the initial sampling time (2 min). Unlike the exhaled breath data, the concentration of PCE in the blood progressively increased over the course of the 2-hr exposure in both exposure groups. The rate of increase was greater in the 500 ppm (Fig. E-4) than in the 50 ppm group (Fig. E-5). Arterial PCE concentrations were not proportional to the inhaled concentration. After the initial rapid uptake phase over the first 30 minutes of exposure, blood levels in the 500 ppm rats were 12 to 17 times higher than 50 ppm rats. Measurement of the cumulative uptake of PCE by the rats was made by accounting for the quantity of unchanged PCE that was exhaled during

the inhalation exposure period. The total cumulative uptake of PCE from the 2-hr exposure to 500 ppm (Fig. E-6) was 28.1 ± 4.3 mg (x ± SD), or 79.9 mg/kg bw. The 2-hr exposure to 50 ppm PCE (Fig. E-7) resulted in a cumulative uptake of 3.9 ± 0.9 mg (x ± SD), or 11.2 mg/kg bw.

IX. STUDIES OF THE PHARMACOKINETICS OF PCE FOLLOWING ORAL ADMINISTRATION IN RATS

Due to the potential for halocarbon exposures in humans to occur due to the ingestion of contaminated drinking water supplies, oral administrations of PCE in rats have also been conducted. Emissions from product manufacturing, usage activities, and spills are thought to be primary sources of halocarbon contamination of water supplies. Recently, the contamination of drinking water supplies by the leakage of solvents from storage tanks and chemical waste dumps has become of significant concern. There are large numbers of solvent and fuel storage tanks in the nation, many at U.S. Air Force facilities. As the majority of these are located underground, leakage of solvents into groundwater supplies can proceed undetected for years. Despite the potential public health significance of halocarbon ingestion from contaminated drinking water supplies, there is presently insufficient information available concerning the systemic absorption and disposition of these and other halocarbons following their oral administration.

One of the primary objectives of this study was to examine the uptake, disposition, and elimination of ingested PCE over a wide range of concentrations. The transition from linear to non-linear kinetics is important in dose-response relationships and in toxic responses. Certain physiological and biochemical processes are linear over a wide range of substrate concentrations. Diffusion of lipophilic halocarbons across cellular membranes is a pertinent example of such a process. Other processes including solubility in the blood and microsomal enzyme metabolism are saturable. When the dose of halocarbon exceeds the capacity of these processes, there is a transition from linear to nonlinear pharmacokinetics. Under such conditions, there is no longer a linear relationship between administered dose-exposure level and blood/target concentrations. Disproportionately high blood/target organ concentrations and accentuated toxicity are characteristic of nonlinear kinetics.

An investigation in to the role of dose level on the pharmacokinetics of ingested PCE has been completed in rats. The results of this study were presented as part of a presentation at the Society of Toxicology meeting in March, 1989 in Atlanta, GA. The reference for this study (also listed in Section M of the Appendix) as presented in the abstract is as follows:

> Ramanathan, R., Muralidhara, S., Dallas, C.E., Gallo, J.M. and Bruckner, J.V. "Influence of the pattern of ingestion on the pharmacokinetics of perchloroethylene (PER) in rats." 28th Annual Meeting of the Society of Toxicology, Atlanta, GA; Toxicologist 9: 78 (1989).

In order to procure repetitive blood samples following administration of single oral bolus doses of halocarbons to unanesthetized rats, an indwelling arterial cannula was surgically implanted prior to the halocarbon exposure. The cannula was tunneled subcutaneously to the back of the animal and exited just

behind the head. The cannula was extruded through a steel spring that was attached to the back of the animal by a harness. After the surgery was complete, the animal was placed into a metabolism cage to recover for 24 hours before halocarbon dosing. PCE was given orally to unanesthetized male Sprague-Dawley rats in an aqueous Emulphor emulsion as a single bolus in doses of 10, 25, 50, and 100 mg/kg. Blood samples were collected from an indwelling carotid arterial cannula for up to 12 hours post administration. The blood samples were analyzed for PCE using a GC-ECD head space technique.

Absorption of PCE from the gut was very rapid, with peak blood levels achieved within 20 minutes of the oral administration for all four dose groups (Fig. E-8). Elimination of the halocarbon in the blood proceeded at a similar rate at each dose level, with the elimination curves roughly parallel up to 12 hours following the oral bolus. When considering all four dose groups, the maximum blood level achieved after the oral bolus (Cmax) was not directly proportional to the dose level. Indeed, the 100 mg/kg dose group demonstrated an average Cmax that was only slightly higher than the mean value for the 50 mg/kg dose group. The Cmax levels for the 10 and 25 mg/kg groups, however, appeared to be somewhat more proportional to the dose level. A comparison of the area-under-the-blood-concentration-time-curve (AUC) between the four dose groups (Fig. E-9) reveals a linear relationship in AUC relative to dosage level between 10 and 100 mg/kg PCE. Fitting an equation for a regression analysis between these four points resulted in an R-square of 0.998, which would be a significantly good fit for a linear equation.

X. DEVELOPMENT OF AN ASSAY FOR HALOCARBON CONCENTRATIONS IN TISSUES

Although knowledge of the deposition of chemicals in target tissues is of major importance in risk assessment, PBPK models have to date been used primarily to forecast uptake and elimination of VOCs from the bloodstream. Blood concentration over time, as a measure of bioavailability, is routinely accepted as an index of the level of chemical in the body, and therefore a representative index of toxic effects. This assumption can be misleading, in that blood concentrations may not be reflective of the concentration of chemical or active metabolite at the local site of effect in a target tissue. Thus, a more logical measure of target organ exposure is the area under the tissue versus time curve for the reactive chemical (Andersen, 1987). Relatively little has been published on the use and validation of PBPK models for prediction of time integrals of tissue exposure to VOCs, because of a paucity of tissue concentration versus time data sets. This lack of a data base is due to the considerable effort required in such studies and to technical problems with quantitation of the volatile chemicals in solid tissues. For example, the only tissue data Reitz et al. (1988b) had to use for validation of their PBPK model were levels of radioactivity measured by Schumann et al. (1982) in the liver and fat of mice and rats at the termination of 6-hour inhalation exposures to ^{14}C-TRI.

It has therefore been a goal of this project to develop an accurate assay for the direct measurement of the parent halocarbon in the tissues of exposed animals. These measurements will be of significant utility for the validation of PBPK models for the prediction of halocarbon pharmacokinetics. Each compartment of the model can therefore be represented (and validated) by a tissue that is sampled in a laboratory experiment. Compartments that are single organ-

specific like the liver, kidney, and brain would be directly represented. Predictions for a physiologically-generalized compartment, such as the one that has been utilized for "poorly perfused" tissues, would be validated by measurements from a representative tissue group, the muscle tissues.

A technique for the analysis of halocarbons in tissues has now been successfully developed and employed in tissue measurement in several experiments. This technique has been compiled in a manuscript and submitted to a peer-reviewed journal simultaneously with the submission of this final report. This manuscript is included as Section F of the Appendix, and the reference is as follows:

> Chen, X.M., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. "Determination of volatile short-chain aliphatic halocarbons in animal tissues." Now being submitted to the Journal of Environmental Pathology, Toxicology, and Oncology (1991).

Extensive analyses have been conducted to determine the efficiency of the measurements for both PER and TET in the following tissues: brain, liver, kidney, lung, fat, heart, and muscle. One-gram tissue samples are placed into 8 ml of chilled isooctane for PER and ethyl acetate for TET. The samples are maintained in an ice bath at all times, even during homogenization. A polytron is used to homogenize the tissue samples. In order to insure that the percent recovery of halocarbon is maximal and reproducible, a specific period of homogenization is required for each tissue. Brain, liver, and fat are the most easily homogenized, requiring only 4 or 5 seconds. Kidney, lung and heart homogenization require 8 to 10 seconds. Muscle is the most difficult, requiring 15 to 20 seconds. Samples are extracted with 8 ml of isooctane for PER or ethyl acetate for TET and vortexed for 30 seconds. Samples are then centrifuged at 3000 rpm for 5 min at 4°C in a Sorvall RC 2-B centrifuge. Twenty μl of the organic phase is withdrawn with a microsyringe and transferred to headspace vials (Perkin-Elmer, Norwalk, CT). One interesting and important methodological finding was that 20 μl was the optimal amount of the organic phase homogenate to add to the headspace vial. Adding aliquots greater than 30 μl resulted in a decrease in the efficiency of recovery of the halocarbon, as determined by comparison to standards with known concentration.

Standards are made and assayed by diluting a calculated amount of pure test chemical in the appropriate solvent. The column used is an 8' x 1/8" stainless-steel column packed with FFAP Chromasorb W-AW (80-10 mesh). Operating temperatures are: injection port, 200°C; electron capture detector, 360°C; column 110°C; headspace control unit, 90°C.

This analytical technique has been used in pilot studies of the tissue disposition of PCE and TET in rats following oral and ia administration. Efficiency of recovery from tissues spiked with the halocarbons ranged from approximately 70% for muscle to nearly 100% for liver and brain. Reproducibility between different spiked tissue samples has been found to be very consistent.

XI. MEASUREMENTS OF PCE IN THE TISSUES OF RATS FOLLOWING ORAL AND INTRAARTERIAL ADMINISTRATION

Groups of male S-D rats (mean body wt = 350 g) were administered PCE via a surgically-implanted carotid artery cannula. As blood flow in the right carotid artery ceases when the cannula is inserted and the vessel ligated, a solution injected through the cannula flows back to the heart and enters the systemic arterial circulation. Thus, this technique allows direct intraarterial (ia) injection. Serial blood and tissue samples have been taken following dosing, in order to characterize uptake and elimination profiles for PCE. Blood and tissue sampling will be carried out beyond the normal 5 to 6 half-lives to ensure that the terminal elimination phase in all tissues is adequately characterized. A minimum of 4 rats were sacrificed at each of the following time points: 1/4, 1/2, 1, 2, 4, 6, 12, 18, 24, and 48 hours after the administration of a single bolus dose. One gram samples of brain, liver, kidney, lung, fat, heart, and muscle were then procured and analyzed as described in Section VII. Profiles of the tissue uptake, disposition, and elimination of PCE following ia and oral administration are compiled in Appendix G.

Absorption of PCE into the tissues following intraarterial (ia) administration was very rapid, as indicated by the significant levels detected in all tissue samples at just 15 minutes following exposure (Appendix G, Fig. 1, or Fig. G-1). The highest levels were achieved in the fat (35.6 μg PCE/g), with levels of 20-26 μg PCE/g in the liver, kidney and brain. By 30 minutes post-administration (Fig. G-2), PCE concentrations in all sampled tissues had declined by 25-50% except for those in the fat, which remained relatively equivalent to that measured after 15 minutes. PCE levels in the fat achieved a peak concentration of 67 μg/g after 1 hour, while PCE concentrations in the other tissues continued to steadily decline (Fig. G-3). Fat levels did begin to decline by 2 hours following exposure (Fig. G-4), while all other tissue groups decreased by more than 60% relative to an hour before. The rate of diminution in non-fat tissue concentrations began to level off by 4 hours (Fig. G-5) and 6 hours (Fig. G-6) post administration. By 6 hours following ia administration, fat levels of PCE had diminished to about 64% of the peak level achieved 5 hours before. Between 6 and 12 hours following PCE administration, levels in non-fat tissues diminished by more than 50%, while fat concentrations decreased by only 9% (Fig. G-7). After another six-hour interval, PCE concentrations in the liver, kidney, heart, and brain remained relatively constant (Fig. G-8). These tissue groups probably have reached an approximate equilibration within the same time frame because they represent the well-perfused tissue groups we have sampled in this study. The muscle tissues, representing the poorly-perfused tissues in this investigation, continued to decline significantly after 18 hours. Tissue measurements taken after 24 (Fig. G-9) and 48 hours (Fig. G-10) appeared to indicate that PCE levels in non-fat tissues were decreasing at a rate of 50% in a 24 hour period, while fat levels demonstrated an 85% decline.

Examination of the tissue concentration-time profiles for ia administration separately for each tissue group sampled indicated definitive similarities and differences between the tissues in PCE disposition and elimination. Profiles for the liver, kidney, and brain (Fig. G-21) were nearly identical. There was a distinct similarity in both the magnitude of PCE concentration and the rate of elimination as reflected by the tissue concentration-time profile for these three well-perfused organs. The lung and the heart (Fig. G-22) also displayed a marked similarity in these parameters. These two tissue groups had PCE concentrations consistently less than in the

tissue concentration-time profiles for the three well-perfused tissues. However, concentrations at the final sampling point (48 hours) converged for these five tissue groups. The muscle tissue profile (Fig. G-22) was similar to that of the heart and lung, but was slightly higher between the 6 and 24 hour points. The fat tissue concentration-time profile (Fig. G-26) was completely different from the other tissue groups. The highly lipophilic nature of PCE obviously resulted in much higher levels in the fat, as well as a much slower rate of decline during the extended elimination phase.

Perhaps the most pressing risk assessment concern presently for exposure to halocarbons by human populations is the potential for oral exposure due to the increasingly reported incidence of contaminated drinking water supplies. Oral exposures were conducted in rats in the present context by administering a 10 mg/kg dose in a single oral bolus. Male Sprague-Dawley rats with a mean body weight of 350 grams were administered the halocarbon with a gavage intubation needle. Sampling times and procedures were identical to those employed for the parallel ia studies.

Absorption of the PCE from the gut was very rapid, as indicated by the significant levels of the halocarbons measured in all tissues after just 15 minutes (Fig. G-11). PCE levels at this initial sampling point were 3-10 times higher after ia administration than after an equivalent dose given orally. Also, the liver and brain levels after oral dosing were actually slightly higher than levels in the fat, a situation which never occurred following ia administration. PCE levels decreased only slightly in most of the non-fat tissues by the 30 minute sampling point (Fig. G-12). Levels of PCE in the lung and fat increased slightly. There was a dramatic increase of PCE in the fat after 1 hour (Fig. G-13), however, while the non-fat tissue levels demonstrated only slight increases or decreases. Indeed, the concentration of PCE in the heart, muscle, lung, and brain had still not begun to decline by the measurements conducted 2 hours after oral dosing (Fig. G-14). All the non-fat tissue concentrations were thus not in a consistent state of decline until 4 hours after oral dosing (Fig. G-15). Peak levels of PCE in the fat were 44.4 $\mu g/g$ at the 6 hour point, while the decline in non-fat tissue levels in that 2 hour interval was minimal (Fig. G-16). Consistent decreases in fat concentration of PCE did not occur until the following six hour intervals, at the 12 hour (Fig. G-17), 18 hour (Fig. G-18) and 24 hour (Fig. G-19) sampling points. By 48 hours after oral dosing with 10 mg/kg of PCE (Fig. G-20), though, the halocarbon could only be detected in the liver (barely) and in the fat.

The analytical method for PER in blood and tissues had the following recovery efficiency per tissue group: liver - 96%; Kidney - 69%; fat - 74%; heart - 76%; lung - 79%; muscle - 80%; brain - 72%; and blood - 105%.

In summary, maximum tissue concentrations (C_{max}) were achieved rapidly following both po and ia administration, within 10 to 60 minutes for all tissues but for PER in fat following oral dosing (6 hours). The nonfat tissue C_{max} values for ia administration were about 4-5 times that of the C_{max} values of the same tissues in po dosed animals. The fat C_{max} for the ia group was 1.5 times that of the po group. The shortest $t_{½}$ for elimination of PER from tissues occurred in the liver, though elimination from the other nonfat tissues was only slightly longer. Due to the highly lipophilic nature of PER, the C_{max} and AUC

values for the fat tissues were substantially greater than the other tissues following both ia and po administration.

The degree of blood perfusion had a significant impact on tissue disposition. Highly perfused organs such as kidney and brain had similar values for C_{max}, AUC, and $t_{½}$ within each administrative route. Poorly perfused and nonlipoidal tissues such as skeletal muscle had a lower C_{max} and AUC than these highly perfused tissues. Relative bioavailability, AUC_{po}/AUC_{ia}, was highest for liver (0.83), with values ranging down to 0.62 for lung and 0.68 for muscle tissue.

XII. TISSUE MEASUREMENTS OF TET FOLLOWING ORAL AND INTRAARTERIAL ADMINISTRATION

Comparisons of the tissue uptake, disposition, and elimination of TET were also made between oral and intraarterial administrations. Groups of male S-D rats (mean body wt = 350 g) were administered TET at 10 mg/kg via a surgically-implanted carotid artery cannula. As blood flow in the right carotid artery ceases when the cannula is inserted and the vessel ligated, a solution injected through the cannula flows back to the heart and enters the systemic arterial circulation. Thus, this technique allows direct intraarterial (ia) injection. TET was administered orally through a gavage needle as a single oral bolus. Serial blood and tissue samples have been taken following dosing, in order to characterize uptake and elimination profiles for TET. Blood and tissues sampling were carried out beyond the normal 5 half-lives to ensure that the terminal elimination phase in all tissues was adequately characterized. A minimum of 4 rats were sacrificed at each of the following time points: 5, 15, 30, 45, 60, and 90 min and 2, 3, and 4 hrs (fat was sampled up to 24 hrs) after the administration of a single bolus dose. One gram samples of brain, liver, kidney, lung, fat, heart, and muscle were then procured and analyzed as described in Section VII. Profiles of the tissue uptake, disposition, and elimination of PCE following ia and oral administration are compiled in Appendix D.

Absorption of the TET was very rapid, with maximum tissue concentrations achieved in all sampled tissues within 30 minutes following oral administration. The maximum tissue concentration achieved after oral TET exposure was 6.07 μg/g of fat (Table H-2). Liver concentrations were nearly as high (4.83 μg/g) following oral dosing as occurred in the fat. The half-lives of TET in liver, kidney, heart, muscle, and brain were similar, at approximately one hour. The $t_{½}$ in fat was 3.6 hours. A similar pattern in elimination was seen following intraarterial administration, with a similar half-life in all of the non-fat tissues (Table H-1). The $t_{½}$ in fat was slightly less than half as long as that in the other tissues. Tissue concentration time profiles in each of the tissues are in the Appendix (Fig. H-1 through H-8), with the oral and intraarterial administrations for each tissue displayed together.

XIII. INTERSPECIES COMPARISONS OF THE PHARMACOKINETICS OF PERCHLOROETHYLENE IN DOGS AND RATS

An important consideration in health risk assessments of halocarbon solvents is the validity of species to species comparisons of the uptake, disposition, and elimination of the chemicals following their ingestion.

Therefore, the relative pharmacokinetics in species of widely varying size was evaluated following the administration of perchloroethylene (PER). The objectives of this study were to: (1) Characterize the uptake, disposition, and elimination of perchloroethylene in two species of wide variation in size, the rat and the dog; (2) Utilize oral and intraarterial administrations of the compound to compare the relative "absorbed dose" in each species; and (3) Evaluate whether interspecies differences in the pharmacokinetics of PER were related to differences in observed toxicity between the species.

The results of these interspecies comparisons for perchloroethylene were presented at the latest meeting of the Society of Toxicology that was held in Dallas, TX in February, 1991. The reference for this abstract (also listed in Appendix M) is as follows:

> Chen, X.M., Dallas, C.E., Muralidhara, S., Tackett, R.L., Bruckner, J.V., and Gallo, J.M. "Interspecies comparisons of perchloroethylene pharmacokinetics following oral and intraarterial administration." 30th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 11: 351 (1991).

Male beagle dogs (5-10 kg), obtained from Marshall Farms, and male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories, were employed in these studies. For the animals intended to receive the ia dose, an indwelling carotid arterial cannula was surgically implanted on the day before the exposure. For obtaining blood samples following compound administration, an indwelling jugular vein cannula was implanted into all the test animals. Both cannulas exited the body of the test animal behind the head, and the animals were allowed to recover from the anesthesia until the following day. Food was withheld during the 18 hour recovery period before dosing.

The rats and dogs were administered PER at doses of 1, 3, or 10 mg/kg. PER was administered as an emulsion in polyethylene glycol (PEG) in a single bolus dose. The ia administration was conducted using the carotid arterial cannula. Oral doses were administered using a gavage needle for rats and a teflon tube for dogs.

Serial 20 μl blood samples were collected from an indwelling cannula in the jugular vein at intervals up to 96 hours following dosing. While rats exhibited only slight neurobehavioral effects following PER ia administration, the dogs receiving the 10 mg/kg ia dose demonstrated a very high degree of central nervous system (CNS) depression. Data for ia administration in dogs are therefore presented only for the 1 and 3 mg/kg doses. PER concentrations in the blood of rats following 1 mg/kg oral administration rapidly declined below the limit of detection (Fig. I-1), so data for oral dosing in rats are shown only for the 10 mg/kg dose.

PER concentrations in the blood samples were analyzed by headspace gas chromatography (GC). The GC was equipped with an electron capture detector and an automatic headspace analyzer. The operating conditions were: headspace sampler temperature, 90°C; column temperature, 110°C; injection port temperature, 200°C; detector temperature, 400°C; column packing, 10% FFAP; flow rate for argon/methane carrier gas, 60 ml/min.

The blood concentration-time data were evaluated by Lagran (M. Rocci and W.J. Jusko) computer programs for the assessment of the appropriate pharmacokinetic model and calculation of relevant pharmacokinetic parameters.

Absorption of PER following oral administration was rapid in rats and dogs, with peak blood levels achieved in both species and at both doses between 10 and 40 minutes (Fig. I-2). The maximum concentration of PER reached in the blood was higher in dogs than in rats for the 10 mg/kg oral dose (Fig. I-3). The terminal elimination half-lives of PER in rats appeared to be shorter than in dogs for each dose and route of administration (Fig. I-6).

Following both oral and intraarterial administration of PER, AUC seemed higher in dogs than in rats though the apparent difference was not sufficient to be statistically significant (Fig. I-5). The interspecies difference was more pronounced following oral administration than after ia dosing.

Following ia doses of 10 mg/kg PER, dogs exhibited severe cardiotoxic symptoms and central nervous system (CNS) depression (including unconsciousness), while rats demonstrated only slight, transient neurobehavioral effects. No CNS effects were observed in either species following po administration of PER at either dose. The blood level data will be utilized to evaluate the utility of physiologically-based pharmacokinetic models in conducting interspecies extrapolations of pharmacokinetic data.

In summary, pharmacokinetic parameters for the oral and intraarterial administration of PER indicate that the uptake of PER following ingestion was relatively higher in dogs than in rats (Table I-1). Higher peak blood concentrations of AUC in dogs relative to rats following an equivalent exposure would indicate a higher bioavailability (Fig. I-7). However, the interspecies pharmacokinetic differences in most cases was not statistically significant.

A difference in bioavailability would be expected to result in interspecies differences in target organ concentrations and subsequent toxicity following equivalent doses in the two species. While there were interspecies differences in central nervous system depression and cardiotoxicity following intraarterial administration, there were no observable differences in toxicity between rats and dogs following oral exposure.

XIV. INTERSPECIES COMPARISONS OF THE PHARMACOKINETICS OF INGESTED TETRACHLOROETHANE

In parallel to the studies of perchloroethylene, the relative toxicokinetics between species of wide variation in size was evaluated following tetrachloroethane (TET) ingestion. Male Sprague-Dawley rats and beagle dogs were administered TET as doses of 10 or 30 mg/kg. The halocarbon was administered in polyethylene glycol (PEG) in a single bolus either orally (po), or by intraarterial administration (ia) through an indwelling carotid arterial cannula. Blood samples were collected from an indwelling cannula in the jugular vein at intervals up to 48 hours following administration, and the halocarbon concentrations analyzed by headspace gas chromatography. The terminal elimination half-lives of TET in dogs were significantly longer than in rats for both routes of administration and for both po doses. Bioavailability, peak blood

levels and the area-under-the-blood-concentration-time curves were also higher in dogs relative to equivalent doses in rats po and ia. These results show that there are significant species differences in the toxicokinetics and bioavailability of ingested TET, and that further evaluations are needed for making interspecies comparisons of toxicokinetic data for halocarbons.

The results of this study were presented at the 11th annual meeting of the Society of Environmental Toxicology and Chemistry in November of 1990. The reference for this study (also listed in Appendix M) as presented in the abstract is as follows:

> Dallas, C.E., Chen, X.M., Muralidhara, S., Tackett, R.L., Bruckner, J.V., and Gallo, J.M. "Interspecies comparisons of the toxicokinetics and bioavailability of ingested tetrachloroethane." 11th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Washington, DC; Global Environmental Issues: Challenge for the 90s: 176 (1990).

Male beagle dogs (5-10 kg), obtained from Marshall Farms, and male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories, were employed in these studies. For the animals intended to receive ia administrations of the test compounds, an indwelling carotid arterial cannula was surgically implanted the day prior to the exposure. for procuring blood samples following halocarbon administration, an indwelling jugular vein cannula was implanted in all the test animals. Both cannulas exited the body of the test animal behind the head, and the animals were allowed to recover from anesthesia until the following day. Food was withheld during the 18 hr recovery period before dosing.

The rats and dogs were administered a single bolus dose of either 10 or 30 mg/kg TET, using polyethylene glycol (PEG) as a dosage vehicle. Both oral doses were administered using a gavage needle for rats and a teflon tube for dogs. The ia administration was conducted using the carotid arterial cannula. While rats exhibited no neurobehavioral effects following TET administration, the dogs receiving the 10 mg/kg ia dose demonstrated a very high degree of central nervous system (CNS) depression. Data for ia administration in dogs is therefore presented only for the 10 mg/kg dose.

Serial 20 µl blood samples were taken at selected intervals for up to 48 hrs following dosing. The concentrations of TET in the blood samples were determined by headspace analysis using a Perkin-Elmer Sigma 300 gas chromatograph equipped with an electron capture detector and an automatic headspace analyzer. The operating conditions for the 6-ft x 1/8-inch stainless steel column were: headspace sampler temperature, 100°C; injection port temperature 200°C; column temperature, 140°C; detector temperature, 400°C; column packing, 3% OV-17; flow rate for argon/methane carrier gas, 60 ml/min.

The blood concentration-time data were evaluated by R-strip (Micromath Scientific Software) and Lagran (M. Rocci and W.J. Jusko) computer programs for the assessment of the appropriate pharmacokinetic model and calculation of relevant pharmacokinetic parameters.

Absorption of TET following oral administration was very rapid in rats and dogs, with peak blood levels achieved in both species and at both doses between 12 and 22 minutes (Fig. J-1, J-7). The maximum concentration of TET reached in the blood was higher in dogs than in rats in all cases, though this difference was only statistically significant for intraarterial administration (Fig. J-2). The half-life of TET was longer in dogs than in rats, and at a high level of significance by intraarterial administration and at both doses given orally (Fig. J-6).

Following both oral and intraarterial administration of TET, AUC was significantly higher in dogs than in rats (Fig. J-5). The bioavailability of TET from oral exposure was higher in dogs than in rats (Fig. J-8). Following equivalent ia doses of 30 mg/kg TET, dogs exhibited severe CNS depression (including unconsciousness) while rats demonstrated no appreciable neurobehavioral effects (Fig. J-4). No CNS effects were observed in either species following po administration at either dose.

There were significant differences in the pharmacokinetics of TET between rats and dogs following oral and intraarterial administration. Higher peak concentrations, higher blood levels over time, and greater bioavailability in dogs relative to rats following an equivalent exposure indicated that a significantly higher "absorbed dose" was received in the dogs. This significant difference in absorbed dose would be expected to result in interspecies differences in target organ concentrations and subsequent toxicity following equivalent doses in the two species.

XV. DEVELOPMENT AND VALIDATION OF PHYSIOLOGICALLY-BASED MODELS IN THE PREDICTION OF HALOCARBON PHARMACOKINETICS

An important goal of the project has been to develop and validate physiologically-based pharmacokinetic (PBPK) models, which will allow accurate prediction of the concentration of halocarbons in blood and tissues over time following inhalation and oral exposure. The pharmacokinetic studies conducted in earlier phases of the project have thus provided a unique data base from which to formulate and test the models. Data from the direct measurements of blood and exhaled breath levels of halocarbon have been compared to simulated values calculated from mass-balance differential equations comprising the model. Thereby, the accuracy of the model has been tested by comparison to observed blood and exhaled breath concentrations.

The current investigation of the uptake and elimination of TRI in rats provided the first available data base for direct measurement of TRI in the exhaled breath and blood during inhalation exposures in rats (See Appendix A, Figures 2 and 3). A PBPK model was therefore developed to describe the disposition of TRI in the rat (Appendix A, Figure 1) using this unique opportunity for comparison of computer simulated values with these direct measurements for validation of the model. The fundamental characteristics of this initial model development were based on the work by Ramsey and Andersen (1984) and their PBPK model for the prediction of the kinetics of inhaled styrene. Model-generated simulations of blood and fat styrene concentrations were in agreement with concentrations measured over a period of hours in rats subjected to a series of vapor levels of styrene. Andersen et al. (1984)

expanded their inhalation model for brominated dihalomethanes to forecast not only the time-course of the parent chemical, but the time-course of two metabolites as well.

In the present model development for TRI, it was assumed that a blood flow-limited model was adequate to characterize the tissue distribution of TRI. Blood flow-limited tissue compartments consider the chemical to be homogeneously distributed throughout the blood, interstitial and intracellular spaces. The differential mass balance equation used for a non-eliminating blood flow-limited organ was:

$$V_i \frac{dC_i}{dt} = Q_i (C_a - \frac{C_i}{R_i})$$

where: V_i = volume of the i^{th} organ
C_i = concentration in the i^{th} organ
Q_i = blood flow for i^{th} organ
C_a = blood concentration
R_i = partition coefficient

Eliminating organs, such as the liver, required a clearance term added to this equation for blood flow-limited compartments. In the case where clearance was constant, the term: $-CL_i/C_i/R_i$ would be employed where CL_i is equal to the intrinsic clearance for the i^{th} organ. For nonlinear clearance, the term $-V_m C_i/(K_m+C_i)$ was added to the appropriate mass balance equation. V_m equals the maximum rate of the elimination process (i.e., metabolism) and K_m equals the concentration at which the rate is half the maximum value.

In the development and validation of physiologically-based pharmacokinetic (PBPK) models in this project for predicting the pharmacokinetics of various halocarbons, an important goal has been to define a single model that has the capability of producing accurate simulations for more than one chemical. Of course, required changes in physicochemical constants are dictated as different chemicals are employed, but the utility of a validated model will be enhanced considerably if additional adjustments in model parameters can be minimized. In the previous year of this project, therefore, a PBPK model was developed that has been tested for its utility in providing reasonably accurate simulations for three different halocarbons: TRI, TCE, and PER.

The PBPK model for inhaled TRI and laboratory data used for the model validation has been published in a peer-reviewed journal. A reprint of the publication is included as Section A of the Appendix. The reference for this publication (also listed in Section F of the Appendix) is as follows:

> Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "The uptake and elimination of 1,1,1-trichloroethane (TRI) during and following inhalation exposures in rats. Toxicology and Applied Pharmacology 98: 385-397 (1989).

Compartmental volumes and organ blood flows were obtained from the literature (Gerlowski and Jain, 1983; Ramsey and Andersen, 1984) and sclaed to 340 g, the mean body weight of rats used in the present study. Partition

coefficients and the metabolic rate constant for TRI were taken from Gargas et al. (1986, 1989), except for the richly perfused tissue:blood and lung:blood partition coefficients, which were assumed to be the same as the liver:blood partition coefficient.

This PBPK model that was validated for TRI inhalation has been evaluated for its utility in predicting TCE levels in rats during and following inhalation exposures. TCE and TRI are interesting test chemicals to compare and contrast since they are so structurally similar, yet differ significantly in metabolic capacity. TRI is only slightly metabolized by the rat, so the impact of liver metabolism on the model simulations was not of great importance. However, since TCE is so highly metabolized, it is essential to incorporate accurate estimates of the Michaelis-Menten parameters into the PBPK model so that the model predictions would correctly estimate metabolic rates. The PBPK model for TCE inhalation was evaluated by comparison to direct measurements of TCE in the blood and exhaled breath of exposed rats. The model and the direct measurements used for validation have been included in a paper now in press at a peer-reviewed journal. The reference for this paper (also included in Section F of the Appendix) is as follows:

> Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M. and Bruckner, J.V.: "Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats." In press, Toxicology and Applied Pharmacology, 1991).

In general, the PBPK model predictions for TCE are in good agreement with the actual data. The prediction of exhaled breath levels during TCE inhalation are in close agreement with the direct measurements of expired TCE at both dose levels. Post-exposure exhaled breath predictions were accurate for the 50 ppm group and slightly underpredicted for the first hour following exposure to 500 ppm TCE (predictions thereafter are accurate). During TCE inhalation exposure, blood level simulations for the 50 ppm group were slightly overpredicted (about 0.1 μg/ml). Post-exposure blood levels were overpredicted during the first hour following exposure to 500 ppm TCE. After the first hour post-exposure, all predicted values are in excellent agreement with the observed TCE concentrations.

The present model incorporated the dynamics between the venous, alveolar and arterial compartments that has been used for a methylene chloride PBPK model (Angelo and Pritchard, 1984, 1987). The representation is appealing in that the venous and arterial blood pools are distinct, and a physiologically realistic membrane transport term (h) controls chemical uptake and elimination at the alveolar-lung interface. The blood flow-limited tissue compartments and the Michaelis-Menten liver elimination are similar to other models on metabolized volatile organic compounds (Andersen et al., 1987). The experimentally measured model parameters, alveolar ventilation, and the inhaled gas concentration, were the only values that were altered for the predictions obtained at the 50 and 500 ppm exposures to TCE. The mean of the measured alveolar ventilation rates for TRI and TCE were used for the oral simulations. First-order absorption rate constants (Ka) and bioavailabilities (f) were empirically estimated, and the value of V_m for TCE was adjusted from the reported value to permit better agreement between observed and predicted TCE blood concentrations.

The PBPK model applications for TCE and TRI were presented together at the latest meeting of the Society of Toxicology and that was held in Atlanta, GA in March, 1989. In addition to the model development and data validation for inhalation exposures, PBPK model simulations for oral administrations of TCE and TRI were also presented. The reference for this abstract (also listed in Section M of the Appendix) is as follows:

> Gallo, J.M., Dallas, C.E., and Bruckner, J.V.: "Physiological pharmacokinetic models for 1,1,1-trichloroethane (TRI) and 1,1,1-trichloroethylene (TCE) in rats following inhalation and oral exposures." 28th Annual Meeting of the Society of Toxicology, Atlanta, GA; Toxicologist 9: 230 (1989).

The PBPK model appears to be a suitable predictor for orally administered TCE or TRI. However, the current absorption rate constant parameters differ for the low and high oral doses, and is not consistent with a linear pharmacokinetic model. Utilizing the same Ka and f values for both doses produces predicted blood TCE concentrations considerably different from the observed concentrations. Resolution of this problem could be achieved by further experimentation (including iv and oral dosing and tissue concentration determinations), and possibly by optimization of the absorption parameters based on the global model.

A third test chemical, PCE, has been employed demonstrating the versatility of the PBPK model for the prediction of halocarbon pharmacokinetics. Observed values from measurements of PCE in the blood and exhaled breath of rats during and following inhalation exposure (see Section VIII of this report and Section E of the Appendix) were used to compare to simulations by the model to establish its accuracy for inhaled PCE. The only changes necessary in the model were the physicochemical constants specific to PCE, and the values measured in the laboratory for the alveolar ventilation and inhaled concentrations specific to the validation experiments. Partition coefficients for PCE for input into the model were obtained from Ward et al. (1988), as well as estimates for the Michaelis-Menten parameters (Table 1, Appendix E).

Model-simulated values for elimination of inhaled PCE in the breath were in close agreement with direct measurements of expired PCE during inhalation exposure at both dose levels. Post-exposure exhaled breath predictions were slightly lower than observed values (Fig. E-2). PCE blood levels were overpredicted during inhalation exposure (Fig. E-3). The computer-simulated levels approached near steady-state more rapidly than did observed values. Post-exposure blood concentrations were either in close agreement or only slightly underpredicted (Fig. E-4). Predictions of the cumulative uptake of inhaled PCE during the course of 500 ppm inhalation exposure were very close to the uptake values calculated from the observed exhaled breath data and monitoring of respiratory volumes (Fig. E-6). Uptake during inhalation of 50 ppm PCE was underpredicted relative to calculated values (Fig. E-7).

The predicted values for the elimination of TRI in the exhaled breath were in very close agreement with the measured values determined in the inhalation exposure to TRI in rats reported in this project (Appendix A, Figure 2 and 3). The simulations were close to the experimentally-observed values during both the uptake and steady-state phases during TRI inhalation, and in the elimination

phase after the termination of exposure. The concentration-time profiles of TRI in the blood were also well-desired by the PBPK model. Only for the near steady-state phase during inhalation exposure to 50 ppm TRI was there a relatively small overestimation (about 50 ng/ml) of the predicted blood levels relative to the observed blood levels. The model predicted a slightly more rapid decline in blood levels postexposure in both groups than was observed during the period of 130-200 min, but levels at subsequent time points were accurately predicted. The ability of the model to accurately predict TRI levels in samples from two different physiological sources in the rat was encouraging in this initial model development effort.

The PBPK model developed in the previous studies for inhaled TRI has also been evaluated for its utility in predicting halocarbon pharmacokinetics following oral administration. The differential mass balance equations comprising the model included the parameters for compartmental volumes, organ blood flows, and partition coefficients as listed in Table I of Appendix A. Input into the model was obtained from the absorption rate-constant controlling TRI uptake into the systemic circulation, and was determined from analysis of blood concentration-time data. Simulated values of the uptake and elimination of a single oral dose of 6 mg/kg of TRI are presented in Figure 11, along with the observed experimental values in rats from the current investigation. Observed and simulated values for TRI oral exposures of 48 mg/kg are shown in Figure 12. An absolute bioavailability (F) of 0.5 and a Ka of 0.05 was employed in these simulations of TRI ingestion. A comparison of these observed and simulated values reveals a reasonable similarity in concentration in the uptake and elimination of ingested TRI. C-MAX, or maximum concentration of TRI attained following ingestion of the compound of the computer simulation, was very close in magnitude to the observed peak arterial concentration observed at both dose levels employed.

In order to improve the accuracy of the model predictions, tissue:blood partition coefficients were estimated using direct measurements of a halocarbon in rat tissues during and following exposure. Male Sprague-Dawley rats were administered 10 mg/kg perchloroethylene (PER) by an indwelling carotid arterial cannula (ia), or inhaled 500 ppm PER for up to 2 hr in dynamic inhalation exposure chambers. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, adipose tissue, and blood were taken during exposure and up to 72 hr post-exposure. Blood and tissue samples were analyzed for PER content using a GC-ECD headspace technique. Maximum tissue concentrations (C_{max}) were achieved for most tissues within 15 min following the termination of exposure, with adipose tissue achieving a C_{max} up to 4 hr following the termination of the inhalation exposure (Table K-1). There were similar terminal elimination half-lives ($t_{½}$) for each of the tissues, except for the fat which exhibited a longer $t_{½}$ value (Table K-2). As comparable tissue $t_{½}$ are consistent with a blood-flow limited model, tissue-blood partition coefficients were calculated for non-eliminating compartments using the ia administration data, by dividing of the area-under-the-tissue-concentration-time curve (AUC) by the blood AUC. For the liver, the first-order metabolic rate constant and tissue PER concentration were also employed in the calculation. The utility of the calculated partition coefficients for PBPK models was evaluated by comparison of the observed tissue concentration-time data for ia and inhalation exposures to PER with predicted

values by the model. Tissue concentration time data were thus demonstrated to provide valuable input for halocarbon PBPK model parameter estimates.

These PBPK simulation studies of PER in tissues were presented at the 30th annual Meeting of the Society of Toxicology held in Dallas, TX in February of 1991. The reference for the abstract of this presentation is as follows.

> Dallas, C.E., Gallo, J.M., Chen, X.M., Muralidhara, S., O'Barr, K., and Bruckner, J.V. "Physiologically-based model parameters estimation from perchloroethylene tissue pharmacokinetics." 30th Annual Meeting of the Society of Toxicology, Dallas, TX; Toxicologist 11: 33 (1991).

Male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories, were employed in these studies. For the animals intended to receive the ia dose, an indwelling carotid arterial cannula was surgically implanted the day before the exposure. The cannula exited the body of the test animal behind the head, and the animals were allowed to recover from anesthesia until the following day. For ia administration, PER was administered at a dose of 10 mg/kg as an emulsion in polyethylene glycol (PEG) in a single bolus dose through the carotid arterial cannula. Inhalation exposures of freely moving animals were conducted in 1.0 M^3 Rochester-type dynamic flow chambers. Each animal was individually housed in wire-mesh exposure cages in the chamber. The 500 ppm perchloroethylene (PER) test atmosphere was generated by vaporization of the compound under a strict air flow- and temperature-controlled schedule. Concentration of PER were monitored by gas chromatography. The animals received up to 2 hours inhalation exposure to 500 ppm PER, and breathed fresh air thereafter until terminal sacrifice.

Groups of animals (n=4) were serially sacrificed (using etherization) following ia dosing at the following time intervals - 5, 10, 15, 30, and 60 minutes, and 1, 2, 4, 6, 12, 24, and 36 hours. Animals were removed from the inhalation chambers at the following intervals during the inhalation exposure: 15, 30, 60, 90, and 120 minutes. Following the termination of PER inhalation, animals were taken for sacrifice at the following intervals: 0.25, 0.5, 1, 2, 4, 6, 12, 24, 36, 48, and 72 hours. Blood samples were obtained by cardiac puncture. Approximately 1 gram each of liver, kidney, brain, lungs, heart, fat, and muscle were quickly removed and placed in 4 ml cold saline. Tissues were homogenized for the shortest possible time intervals, specific for each organ, to reduce the volatilization of the test compound during homogenization. The PER in each sample was then extracted with 8 ml Isooctane. A 20 μl aliquot was placed in an 8 ml headspace vial, which was capped and subjected to controlled temperature and pressure conditions in a Perkin-Elmer HS-6 Headspace Sampler.

Analysis was made of the PER in the vial headspace on an (8' x 1/8") stainless steel column in a Perkin Elmer gas chromatograph with an electron capture detector. The column temperatures were: detector-360°C, column-110-°C, headspace-90°C, injector-200°C. Values were compared to a standard curve, and the tissue concentration corrected for the percent recovery characteristic for each tissue.

Maximum tissue concentrations (C_{max}) were achieved within 15 minutes following the termination of inhalation exposure for all nonfat tissues. C_{max} was achieved within 60 minutes following ia administration and at 4 hours following the termination of PER inhalation.

The rate of elimination of PER was somewhat similar among the various rat tissues sampled following both routes of exposure. The $t_{½}$ of PER in tissues following ia administration was in a narrow range, from 6.6 to 7.8 hours. The range of $t_{½}$ for nonfat tissues following PER inhalation was 5.5 to 7.7 hours, and the fat $t_{½}$ was 9.6 hours. Due to the highly lipophilic nature of PER, the C_{max} and AUC values for the fat tissues were substantially greater than the other tissues following both ia administration and inhalation exposure.

Concentrations of PER in blood (Figs. K-16 and F-24), brain (Figs. K-15 and K-23), kidney (Figs. K-10 and K-18), heart (Figs. K-12 and K-20), and lung (Figs. K-13 and K-21) following ia administration were well simulated by the PBPK model using the partition coefficients calculated from the observed ia tissue concentration data. PER concentrations in fat were slightly overpredicted following the attainment of C_{max}, with close predictions again attained after 18 hours (Figs. K-11 and K-19). PER concentrations were also well simulated in liver (Fig. K-9) and muscle (Fig. K-14), with only a slight underprediction at the beginning of the terminal elimination phase.

For simulation of PER kinetics during and following inhalation exposure, there was a general tendency for underprediction to varying degrees using the PBPK model with the calculated partition coefficients. There were underpredictions during the 2 hour inhalation exposure and up to the terminal elimination phase for brain, heart, liver, fat, blood, and lung. PER concentrations for these tissues in the terminal elimination phase were, for the most part, well predicted. Concentrations of PER in the kidney were fairly well predicted both during and following inhalation exposure.

XVI. MEASUREMENTS OF THE NEUROBEHAVIORAL TOXICITY OF HALOCARBONS

In estimating toxicity from pharmacokinetic data and ultimately using such estimates for interspecies extrapolation, two assumptions are inherent: that the intensity of toxic response from an administered dose depends upon the magnitude of the dose reaching the target tissue; and that equivalent target tissue doses in multiple species produce the same degree of effect. At present, little scientific data exist to support or refute the above assumptions. Since the validation of these assumptions is critical if the results of animal studies are to be confidently used to predict the consequences of human exposures, Dr. Dallas' laboratory is aggressively developing a means of validation using neurobehavioral testing. Mr. Alan Warren, the recent recipient of a Department of Defense Science and Engineering Graduate Fellowship, has been using tests of operant performance to measure the central nervous system (CNS) effects of solvents and subsequently will correlate those effects with the solvent concentration in the target organ, the brain. Two animal species, rats and dogs, will be chemically exposed and operant tested to determine whether equivalent brain concentrations in rats and dogs result in CNS effects of comparable magnitude. Although operant testing will undoubtedly be a valuable tool in the growing field of behavioral toxicology, the present challenge lies in applying

this technology to test the dose dependency of solvent-induced CNS effects, to determine the appropriateness of interspecies extrapolation, and to develop and validate toxicodynamic models.

Significant insight into the utility of operant technology, both applied and theoretical, has been gained. Thus far, our experiences have given us confidence in operant technology as a means of validating the assumptions common to the use of pharmacokinetic data and interspecies extrapolation.

The operant testing system, consisting of a computer-controlled and monitored test cage for rodents, is located in the inhalation toxicology laboratory. The test cage has been placed inside a dynamic flow inhalation chamber which is monitored with a Miran 1B2 infrared spectrometer equipped with an external data logger. The Miran 1B2 allows us to very rapidly reach target exposure concentrations inside the inhalation chamber and to maintain concentrations within a very narrow range. The spectrometer is calibrated with a closed-loop calibration system and measurements taken with the Miran 1B2 are compared with gas chromatographic analyses of chamber air.

To this point, only perchloroethylene has been used, but we intend to contrast the CNS effects of perchloroethylene with those of tetrachloroethane. In the case of perchloroethylene, experiments have been performed in an effort to define a dose range that will result in a continuum of increasing behavioral decrement that can be correlated with brain concentrations. All data presented in this progress report are from dose-finding experiments.

Exposure via inhalation was originally proposed, and has been the route of chemical administration most explored. Prior to exposure, the food intake of male, Sprague-Dawley rats is restricted and the rats are trained to lever press for an evaporated milk reinforcement on a fixed ration-20 schedule. Once the rat's operant response rate stabilizes for multiple training sessions, the rat is considered "trained" and ready for exposure. The trained rat is placed in the operant test cage, given 15 minutes to establish a baseline response rate, and is then exposed to a steady concentration (500, 1000, 1500, 2000, or 3000 parts per million) of perchloroethylene for the duration of the operant test. It was expected that response rates would decrease slowly as the concentration of perchloroethylene in the brain increased. However, the response rates of animals drastically decreased upon initiation of exposure, in most cases to zero responses per 5 minutes (See Fig. L-1). Response rates remained depressed for an unpredictable period of time, and the rats either began responding again or in some cases remained non-responders for the duration of the test (See Figs. L-1 and L-2). In some cases, the rats responded at a rate higher than baseline once they recovered from the response decrement brought about by the initiation of exposure (See Fig. L-3). The response rates were concentration dependent as seen in a comparison of the areas under the time-response curves during exposure to 500 parts per million (See Fig. L-4).

The abrupt decline in response rate occurred immediately upon the initiation of exposure at concentrations above the odor threshold but below those thought irritating to rat mucous membranes. Thus, the absence of responding was thought to be olfactory mediated, although it is recognized that irritation may occur once higher chamber concentrations are reached. This line of thinking was

shared by Moser and Balster (1985), in which they state that "a possible mechanism for the behavioral disruption we observed during solvent exposure is the odor and irritant properties of these compounds."

Since the sensory-mediated disruption of responding prevented the correlation of response rates to chemical levels in the brain, the search for a solution was begun. Two solutions have been identified. The first is to render the rats anosmic by bathing the animal's olfactory mucosa with a 5% zinc sulphate solution. Although anosmia may be induced by other techniques, the use of zinc sulphate is advantageous in that it does not introduce secondary, nonsensory effects which could confound interpretation of operant test data. The second possible solution is to use an alternative means of chemical administration that does not invoke sensory responses. Gastric infusion (GI) is currently being explored and appears to be a feasible alternative. It is thought that intraarterial (IA) administration could also prove useful. In addition to circumventing the sensory-mediated problem, GI and IA administration simulate the ingestion of contaminated drinking water and eliminate the uncertainty of absorbed dose, respectively.

One should not assume that the use of gastrically cannulated rats in an operant test system is without difficulties. One obstacle is the maintenance of the cannulas' integrity for the duration of the rat's surgical recovery, operant training period, and operant test, all of which may encompass 12 to 14 days. To prevent the rat from destroying the cannula, a small diameter spring, one inch long, is mounted on the animal's back running lengthwise from the base of the neck toward the tail. The cannula which exits from the base of the neck is protected inside the spring. A metal connector is attached to the end of the gastric cannula and exits the spring to provide a point of connection for the infusion tubing.

An additional problem is the protection of the infusion tubing once joined to the connector. The solution to this problem involves suspending a small diameter spring, approximately 8 inches in length, from the top of the operant testing cage to the point where the infusion tubing connects with the gastric cannula. The spring length is just long enough to allow the rat full range of motion within the operant testing cage. Between the back-mounted spring and the one suspended from the cage top, the entire length of the cannula and infusion tubing is safeguarded from the rat.

A third problem is the preoccupation of the rat with the cannula and infusion tubing during operant training and the operant test. This diverts the rat's attention away from depressing the lever to obtain the reinforcement. It is hoped that by training cannulated animals with the infusion tubing intact, the preoccupation will wane. An assessment is also being made of the effectiveness of cannulating animals after their training has taken place in hopes that they will be so engrossed in obtaining reinforcements, that the infusion tubing will largely be ignored. Our experience indicates that when cannulating after training has taken place, memory loss due to the anesthetic is possible. This may call for the experimental use of multiple anesthetics to identify one that does not interfere with recall of the learned relationship between level press and reinforcement.

The administration of test chemicals via inhalation, GI, and IA infusion are all therefore being employed during operant testing. This will allow us not only to meet our original goal of testing common assumptions, but also to draw conclusions about the role of exposure route in determining the degree and time-course of CNS toxicity. Additionally, the ability to administered test chemicals by multiple routes will enable us to better utilize existing pharmacokinetic data generated using these administration routes.

XVII. COLLABORATIVE ARRANGEMENTS

As Principal Investigator, Dr. Cham E. Dallas has been responsible for the overall supervision of the project. Dr. Dallas has personally conducted the inhalation exposures, including experiments with TCE, TRI, DCE, and PER. He has also developed the novel mathematical approaches to the analysis of the respiratory monitoring data for the halocarbon inhalation studies (see Appendix A). Dr. James V. Bruckner, as Co-Principal Investigator, has been responsible for the design and conduct of the pharmacokinetic studies of halocarbon ingestion. Dr. James Gallo has had the primary responsibility for the development and validation of the physiologically-based pharmacokinetic model from the experimental studies. Dr. Peter Varkonyi has also helped in these PBPK model validations, especially with the use of tissue validation data. Dr. R. Ramanathan has participated in the analysis of blood samples for halocarbon uptake and disposition from the test animals. This effort was in conjunction with an EPA project on the effect of exposure route on the toxicity of volatile organics, which is intended for use in setting drinking water standards. Mr. S. Muralidhara has conducted the animal surgery required, analytical determinations of blood samples, oral exposures to halocarbons, and data analysis. Miss Elizabeth Lehman was an undergraduate chemistry student who assisted in the conduct of the laboratory studies, glassware washing, and record keeping. Dr. Randall Tackett was involved in the pharmacological and pharmacokinetic studies using the dog as an animal model. Dr. Tom Reigle has had extensive operant behavior testing experience, and has already provided valuable assistance in the selection, purchase, and workup of the appropriate testing equipment that can be used for both rats and dogs.

Dr. Xiao Mei Chen (XMC) has served as a full-time postdoctoral associate on the project. She was successful in her work in the development of the assay for the measurement of halocarbons in the tissues of exposed animals (see Appendix F), and has conducted these tissue measurements thus far for ia and po exposures for PER and TET. Mr. Alan Warren, the recent recipient of a Department of Defense Science and Engineering Graduate Fellowship, is a doctoral student who is using tests of operant performance to measure the central nervous system (CNS) effects of solvents and subsequently will correlate those effects with the solvent concentration in the target organ, the brain. Very useful technical information and counsel on the development of the PBPK models and the analysis of pharmacokinetic data in halocarbon inhalation exposures has been received in consultation with Drs. Melvin Andersen, Harvey Clewell, and Michael Gargas at the Biochemical Toxicology Branch, Toxic Hazards Division, Harry G. Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base.

XVIII. STATEMENT OF SIGNIFICANCE ADDITIONAL STATEMENTS SECTION

Comparison of the pharmacokinetic results for the inhalation of TRI, DCE, and TCE in rats provides insight into the relative importance of the roles of metabolism and volatility in the uptake, disposition, and elimination of these halocarbons. Detailed discussions of the results are presented in the papers published, in press, and prepared for submission and included with this report for TRI (Appendix A), TCE (Appendix B), and DCE (Appendix C). DCE and TRI have a similar propensity for volatility as reflected by blood:air partition coefficients of 5.0 and 5.8. However, the two halocarbons are quite different in their propensity for metabolism. Studies of inhaled DCE excretion patterns have indicated that sufficiently high doses exceed the metabolic capacity of exposed animals (Andersen and Jenkins, 1977; McKenna et al., 1978; Jones and Hathaway, 1978). Pharmacokinetic studies of TRI in rats (Schumann et al., 1982) and humans (Nolan et al., 1984), though, have clearly demonstrated that this halocarbon is not metabolized to a significant extent in either species. Indeed, with the high volatility and relative lack of metabolism, the majority of the inhaled TRI was found to be eliminated unchanged in the breath of rats and man in a linear pattern related to dose. In the present study in rats, DCE and TRI were found to have a very similar magnitude of uptake of the inhaled dose over time (adjusted for differences in inhalation concentration). For instance, DCE uptake for 100 ppm exposure for 2 hours was 10 mg/kg, while TRI uptake at half that dose (50 ppm exposure) for 2 hours was 6 mg/kg. By contrast, total uptake of TCE for a 50 ppm exposure for 2 hours was 8.4 mg/kg. Like DCE, TCE has been found to be significantly metabolized to various metabolites in rats (Stott et al., 1982). For the inhalation of TCE, this dose-dependent metabolism was calculated to be saturable at approximately 65 ppm (Filser and Bolt, 1979). Unlike DCE and TRI, however, TCE has a relatively high blood:air partition coefficient (21.9), which indicates that volatility is less of a factory for TCE relative to these halocarbons. As DCE and TRI uptake were similar and both were lower than for TCE, it seems that the characteristic volatility of the halocarbon is relatively more significant to the total uptake during inhalation exposures than the role of metabolism.

Metabolism was a key factor, however, when considering the systemic disposition of inhaled halocarbons as reflected by the blood levels of inhaled TCE, TRI, and DCE. For all three halocarbons, substantial levels of the inhaled compounds were present in the blood within minutes after the initiation of exposure. Each inhaled halocarbon demonstrated a rapid uptake phase that encompassed approximately the first thirty minutes of inhalation exposure. Once a near steady-state was achieved after this rapid uptake phase, however, a distinct pharmacokinetic difference exists between the three halocarbons related to the propensity for metabolism by the compound. Near-steady-state levels for TCE and DCE were not proportional to the inhaled concentration, while steady-state levels for inhaled TRI were proportional to the inhalation exposure level. Indeed, TCE blood levels continued to increase progressively throughout the inhalation of 500 ppm TCE, and were 25-30 times greater than in 50 ppm-exposed rats. As DCE and TCE are significantly metabolized and TRI is not, this indicates that metabolism (and the saturation of metabolic capacity) is still a very important factor in systemic disposition patterns of inhaled halocarbons.

In view of the aforementioned relationships, an interesting finding in the present investigation is the data involving the measurement of the respiratory elimination of the halocarbons in the rat. As in the determinations of

halocarbon uptake in the blood, elimination of TCE, TRI, and DCE in the breath increased rapidly during the first thirty minutes of exposure until a near steady state equilibrium was reached thereafter. Unlike uptake in the blood, however, near steady-state exhaled breath levels of all three halocarbons were proportional to the inhalation exposure concentration. This is not surprising when considering TRI, a halocarbon which does not undergo significant metabolism and also exhibits linear pharmacokinetic in systemic uptake in the blood. For both DCE and TCE, though, respiratory elimination of these well-metabolized halocarbons remained linear to the inhaled dose regardless of disproportionate uptake occurring simultaneously in the blood. It is apparent that the characteristic high volatility of halocarbons is a critical factor in determining the relative elimination of the compounds in the rat, at least in short-term inhalation exposures.

Determinations of elimination of halocarbons in the breath of rats has also revealed a finding that is of significance to the interspecies extrapolation of pharmacokinetic data from rats to humans. In addition to their structural similarity yet definitive differences in metabolism, TCE and TRI were selected as test chemicals in the present investigation because there is a unique pharmacokinetic data base available for these two halocarbons in humans, Therefore, a comparison of the previously unavailable direct measurements in the rat in the present investigation could be made with these pharmacokinetic determinations conducted in humans. A very interesting finding from this comparison is that the concentration of TCE and TRI in the exhaled breath of rats was very similar to that measured in humans, adjusting for differences in the exposure concentration employed. For TRI, there was exhaled breath data available during inhalation exposures in humans (Nolan et al., 1984), as well as following exposure. The exhaled breath levels after 1.5 hr of exposure to 35 and 350 ppm of TRI were 0.14 and 1.28 μg/ml, respectively. Assuming a linear scale-up to a 50 and 500 ppm exposure (0.2 and 1.83 μg/ml, respectively), these exhaled breath levels in humans are very similar to exhaled levels measured after 1.5 hr of exposure in the present study in rats (0.21 and 2.16 μg/ml, respectively). In both the human study and the rat study reported here, TRI elimination in the breath was proportional to the exposure concentration.

Exhaled breath determinations of TCE in humans have centered on measurements conducted following the termination of exposure. The exhaled breath of exposed workers has been monitored for expired TCE following inhalation exposure as a non-invasive method for indicating the magnitude of prior exposure to the solvent (Stewart et al., 1970, 1974). Measurements of TCE in both the blood and exhaled breath of workers following TCE inhalation have been made in studies of the effect of workload (Monster et al., 1976) and repeated exposure to TCE (Monster et al., 1979) on subsequent pharmacokinetics of the inhaled solvent. Accounting for differences in exposure concentration, the post-exposure exhaled breath levels of TCE from these studies in humans were similar in magnitude to the values of TCE eliminated in the exhaled breath of rats following inhalation exposure in the current investigation. For instance, Stewart et al. (1974) found human exhaled TCE levels of 0.70 and 0.28 ppm at 30 and 120 min, respectively, after termination of 20 ppm TCE inhalation for 3 hrs. Scaling-down the 50 ppm data in rats in the present study at these time points would yield 0.92 and 0.28 ppm, respectively. Apparently due to its relatively high hepatotoxicity, even after brief inhalation exposure in animals, there have not

been pharmacokinetic determination of DCE made in humans. it is evident that with halocarbons for which volatility is such a critically important characteristic, such as TCE and TRI, elimination in the breath of rats and man follows a similar pattern. These results indicate that the rat may be a potentially useful model for evaluating the respiratory elimination of inhaled volatile halocarbons in man.

Similarities in the anatomy and physiology of mammalian species make interspecies extrapolations (i.e. animal scale-up) possible (Dedrick, 1973; Boxenbaum, 1984). A model developed in one species may be scaled to allow prediction of chemical concentrations in other species without the collection of additional experimental data. Model parameters such as tissue volumes and blood flows can be scaled based on allometric relationships which are functions of animal weight. The ability to scale physiological models validated in animals to humans is a powerful tool to obtain predictions of tissue chemical concentrations in humans.

Confidence in the use of scaled animal-based models to predict the kinetics of chemicals in humans can be gained only if the animal model can accurately predict chemical concentrations measure in the animal. the model utilized in the present investigation predicted blood and exhaled breath concentrations of TRI TCE, and DCE reasonably well during and following inhalation exposures in the rat, as determined by comparison with direct measurements conducted in studies on this project. The same model was able to predict peak blood levels and the systemic elimination of TRI following oral exposure with reasonable accuracy, indicating potential utility of the approach for the simulation of the ingestion of halocarbons. Further, the model has been further evaluated to serve as a reliable predictor for halocarbon tissue concentrations. Although it has formerly been common practice to calculate risk estimates on the basis of administered dose-toxicity/tumor incidence data, it is now recognized that the internal, or target organ dose is a more accurate and direct determinant of the magnitude of injury. The dose of chemical actually reaching a target organ is dependent upon kinetic processes which may vary considerably with the administered dose, route of exposure, and animal species. Thus, recognition and use of pharmacokinetic data can substantially reduce uncertainties inherent in the route-to-species extrapolations often necessary in risk assessment (Gehring et al., 1976; Clewell and Andersen, 1985; NRC, 1987).

A basic tenet of toxicology is that of the dose-response relationship (i.e., the magnitude of toxic effect is a function of chemical dose). The concept of dose is now being refined, as it is recognized that the amount of chemical absorbed systemically (i.e., internal dose) can vary significantly with route of exposure and with animal species. Blood levels over time following exposure have been accepted historically as indices of internal dose, but they often may not accurately reflect concentrations of active chemicals at local sites of action in tissues. Thus, the most logical and precise measures of dose are time integrals of target organ concentrations of chemicals. As alluded to previously, there are a paucity of such data sets for VOCs, due largely to the technical difficulties and inordinate time involved in analysis of these highly volatile compounds. The sensitive technique developed in the studies presented here (Appendix F), which allows rapid, reproducible measuremnt of different halocarbons in a variety of tissues should be useful to generate comprehensive

tissue dose-response data for VOCs of concern. Recognition and utilization of such information can substantially reduce uncertainties inherent in toxicity and carcinogenicity risk assessments.

The PBPK model used in the current investigation accurately predicted the time-courses of halocarbon concentrations in the blood and tissues of rats and dogs during and following exposure to these environmental contaminants. The model is similar to those of Ramsey and Andersen (1984) and Angelo and Pritchard (1984). Our PBPK model differs in that it includes a separate lung tissue compartment and a lung:alveolar mass transfer coefficient, which describes the bidirectional transfer of TCE across the alveolar membrane. It is only necessary to alter the experimentally determined inhaled concentration and minute volume in order to obtain simulations of halocarbon kinetics under different inhalation exposure scenarios. Metabolic saturation, manifest by the progressive, disproportionate increase in blood levels in the high-dose animals, was accurately forecast. There was also good agreement between predicted and observed blood and breath levels during most of the postexposure period. Previous investigators, including Fisher et al. (1989), have had the use of very limited experimental data sets for assessing the precision of their model predictions. It is anticipated that a model thus validated will have the following important applications: (a) prediction of blood and target organ levels following inhalation and ingestion of halocarbons, in the absence of data; (b) interspecies extrapolations (i.e. scale-up from small to large laboratory animals and ultimately to man).

One of the benefits from the completion of these studies of TCE, TRI, and PCE in this project is that there is a unique data base with pharmacokinetic determinations of the uptake and elimination of these two halocarbons in humans. Therefore, the PBPK model validated in experiments in this project can then be evaluated for its ability to extrapolate to man, using comparisons to these previously published values for human halocarbon pharmacokinetics. Such a validation would establish greater merit in employing the established model in making extrapolations of pharmacokinetic data from test animal species to man, or to predict blood and tissue levels of halocarbons in man in the absence of experimental data.

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APPENDIX A

REPRINT OF PAPER PUBLISHED IN
TOXICOLOGY AND APPLIED PHARMACOLOGY

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "The uptake and elimination of 1,1,1-trichloroethane (TRI) during and following inhalation exposures in rats. Toxicology and Applied Pharmacology 98: 385-397 (1989).

TOXICOLOGY AND APPLIED PHARMACOLOGY 98, 385–397 (1989)

The Uptake and Elimination of 1,1,1-Trichloroethane during and following Inhalation Exposures in Rats[1,2]

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Received July 1, 1988; accepted January 16, 1989

The Uptake and Elimination of 1,1,1-Trichloroethane during and following Inhalation Exposures in Rats. DALLAS, C. E., RAMANATHAN, R., MURALIDHARA, S., GALLO, J. M., AND BRUCKNER, J. V. (1989). *Toxicol. Appl. Pharmacol.* 98, 385–397. The pharmacokinetics of 1,1,1-trichloroethane (TRI) was studied in male Sprague–Dawley rats in order to characterize and quantify TRI uptake and elimination by direct measurements of the inhaled and exhaled compound. Fifty or 500 ppm TRI was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 325–375 g. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently both during and following TRI inhalation and analyzed for TRI by gas chromatography. Respiratory rates and volumes were continuously monitored during and following exposure and were used in conjunction with the pharmacokinetic data to characterize profiles of uptake and elimination. TRI was very rapidly absorbed from the lung, in that substantial levels were present in arterial blood at the first sampling time (i e , 2 min) Blood and exhaled breath concentrations of TRI increased rapidly after the initiation of exposure, approaching but not reaching steady state during the 2-hr exposures. The blood and exhaled breath concentrations were directly proportional to the exposure concentration during the exposures. Percentage uptake of TRI decreased 30–35% during the first hour of inhalation, diminishing to approximately 45–50% by the end of the exposure. Total cumulative uptake in the 50 and 500 ppm groups over the 2-hr inhalation exposures was determined to be 6 and 48 mg/kg body wt, respectively. By the end of the exposure period, 2.1 and 20.8 mg, respectively, of inhaled TRI was eliminated from rats inhaling 50 and 500 ppm TRI. A physiological pharmacokinetic model for TRI inhalation was utilized to predict blood and exhaled breath concentrations for comparison with observed experimental values. Overall, values predicted by the physiological pharmacokinetic model for TRI levels in the blood and exhaled breath were in close agreement with measured values both during and following TRI inhalation.

1,1,1-Trichloroethane (TRI), also known as methyl chloroform, has been used in large quantities for decades in industry as a solvent and metal degreasing agent. Other applications include its use in adhesives, spot removers, aerosols, and water repellents. The toxicity of TRI is considered to be of a relatively low order of magnitude, with depression of the central nervous system (CNS) (Torkelson

[1] Research sponsored by U.S. EPA Cooperative Agreement CR 812267 and the Air Force Office of Scientific Research, Air Force Systems Command, USAF, under Grant Number AFOSR 87-0248. The U.S. Government's right to retain a nonexclusive royalty-free license in and to the copyright covering this paper, for governmental purposes, is acknowledged.

[2] Presented at the 26th Annual Meeting of the Society of Toxicology, Washington, D.C., February 1987.

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0041-008X/89 $3.00

and Rowe, 1981; Kleinfeld and Feiner, 1966; Stewart, 1968) and cardiac arrhythmias (Dornette and Jones, 1960; Reinhardt *et al.*, 1973; Herd *et al.*, 1974) the major effects seen after high doses in animals and humans. Hepatic and renal toxicity have been demonstrated only after very high acute doses in animals (Plaa and Larson, 1965; Klaassen and Plaa, 1966, 1967; Gehring, 1968). Historically, human exposures to TRI have been of greatest significance in industry and other occupational settings, where exposures are primarily by inhalation. Workers are routinely exposed to TRI vapors in open or closed (i.e., recirculating) work environments. Employees may be inadvertently exposed to high concentrations when there has been a spill or equipment malfunction.

Studies of the pharmacokinetics of inhaled solvents such as TRI are playing an increasingly important role in toxicology. Knowledge of the uptake, disposition, and elimination of these chemicals is quite useful in health risk assessments. There is presently little kinetic data available involving direct measurements of TRI in laboratory animals during inhalation exposures. The fate of ^{14}C-TRI has been investigated following the termination of single 6-hr 150 or 1500 ppm inhalation exposures in rats and mice (Schumann *et al.*, 1982a). By 72 hr postexposure, 87–98% of the total recovered radioactivity was eliminated as unchanged TRI in the expired air. Respiratory elimination and metabolism of TRI remained approximately the same after TRI inhalation exposures were repeated 5 days/week for 18 months (Schumann *et al.*, 1982b). The fraction of the total inhaled dose which is eliminated during ongoing inhalation exposures, however, has not been delineated in laboratory animals. Likewise, the rate and magnitude of uptake have not been quantified over time during the course of TRI inhalation exposures in animals. It was necessary, for example, for Schumann *et al.* (1982a) to base estimates of pharmacokinetic parameters for rats on an assumed constant uptake of 60% of inhaled TRI over 6 hr of exposure.

Therefore, an objective of the current investigation was to provide accurate measurements of the respiratory uptake and elimination of TRI during inhalation exposures. Inhaled and exhaled breath concentrations were monitored at frequent intervals in rats both during and following TRI inhalation, as were the minute volume and respiratory rate. Blood levels of TRI were monitored concurrently, so systemic uptake and elimination could be correlated with the respiratory measurements. The exhaled breath and blood TRI concentrations were then utilized to assess the accuracy of values predicted by a physiologically based pharmacokinetic model for TRI inhalation.

METHODS

Animals. Adult, male Sprague–Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light–dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. They were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided *ad libitum*. The rats were used after at least a 14-day acclimation period, at which time they were approximately 12 weeks old and their body weight ranged from 325 to 375 g. Solvent exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test material. 1,1,1-Trichloroethane, 98.3% minimum purity, was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). The purity of the chemical during the conduct of the study was verified by gas chromatography to be slightly less than 99%.

Animal preparation. An indwelling carotid arterial cannula was surgically implanted into each animal. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml) acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). The cannulated animals were maintained in a harness and pulley system that allowed relative freedom of movement in metabolism cages during a 24-hr recovery period.

Inhalation exposures. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneve, Switzer-

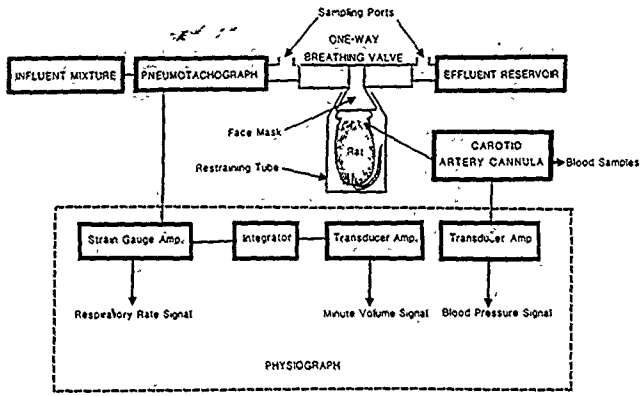

FIG. 1. Schematic diagram of the inhalation exposure system. An unanesthetized rat in the restraining tube inhaled TRI through the one-way breathing valve attached to the face mask. TRI was inhaled from the influent mixture gas sampling bag and exhaled into the effluent reservoir bag. Inhaled and exhaled breath samples were taken from their respective sampling ports and arterial blood samples from an indwelling cannula. The rate and volume of respiration were monitored on a physiograph. For sake of clarity, the breathing valve, gas sampling bags, and other components are not drawn to scale.

land). A face mask designed to fit the rat was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. A miniaturized one-way breathing valve (Hans Rudolph, Inc., St. Louis, MO) was attached to the face mask so that the valve entry port was directly adjacent to the nares of the test animal. The dead space of the valve was 0.5 ml. The valve was designed so that the negative pressure generated by the animal's inspiration pulled the inhalation diaphragm open and the exhalation diaphragm closed against its seat. Upon expiration, the positive pressure generated within the device pushed the exhalation diaphragm open and the inhalation diaphragm closed against its seat. This established separate and distinct airways for the inhaled and exhaled breath streams with no significant mixing of the inhaled and exhaled air. The use of such a device for pharmacokinetic studies of inhaled halocarbons in small animals has been described in detail (Dallas *et al*., 1986). Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. A known concentration of TRI was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of the test chemical into the bag filled with air. Uniform dispersion of the vapor was ensured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, a three-way connector, the breathing valve, and an empty 70-liter gas collection bag (Fig. 1). The latter bag served as a reservoir to collect exhaled gas. Thereby, a closed system was maintained to prevent release of the agent into the laboratory. TRI inhalation exposures were initiated only after stable breathing patterns were established for the cannulated animals in the system. Just before the initiation of exposure, the solvent vapor was first drawn out of the gas sampling bag by an air pump attached to the three-way connector. In this manner, the animal was assured of being subjected at the very start of the exposure to a TRI concentration equivalent to the target concentration in the bag, without significant dilution from dead space air in the system. The test animals then were subjected to 2-hr TRI inhalation exposures. During this period and for up to 4 hr afterward, inhaled and exhaled breath samples were taken from the sampling ports at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for TRI content by gas chromatography.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored. The respiratory monitoring was conducted according to the methods previously used in solvent exposure studies by this laboratory (Dallas *et al*., 1983, 1986). The airflow created by the animal's inspiration was detected by a pneumotachograph located in the inhaled airstream be-

tween the influent bag and the breathing valve. The signal from the pneumotachograph and accompanying transducer was employed in recording the number of respirations per minute (f) in one channel of a physiograph. This signal was then intergrated over a 1-min interval to yield the volume of respiration per minute, or minute volume (V_E). A value for the average tidal volume (V_T) during that 1-min interval was determined by dividing V_E by f for that minute. An average value for these parameters for individual animals was obtained by averaging the measurements taken at 10-min intervals during the 2-hr exposure. The mean ± SE of these values for the 500 ppm exposure group ($n = 6$) were $V_E = 236.3 \pm 22.9$ ml/min; $f = 135.3 \pm 6.6$ breaths/min; and $V_T = 1.74 \pm 0.18$ ml. The mean ± SE of these values for the 50 ppm exposure group ($n_1 = 6$) were $V_E = 252 \pm 14.7$ ml/min; $f = 129.5 \pm 13.5$ breaths/min; and $V_T = 1.96 \pm 0.1$ ml.

Since the V_E and the TRI exhaled breath concentration at each sampling point were measured, subtraction of the quantity of TRI exhaled from the amount inhaled yielded an approximation of the quantity of TRI taken up each sampling period (cumulative uptake, or Q_{upt}).

$$Q_{upt} = (C_{inh} V_E t) - (C_{exh} V_E t), \quad (1)$$

where C_{inh} is the inhaled concentration; V_E and C_{exh} are the minute volume and exhaled breath measurements, respectively, made at each time point; and t is the interval of time between sampling points (every 10 min for Q_{upt}). The successive Q_{upt} values are summed to determine cumulative uptake over the 2-hr exposure.

Determination of the cumulative elimination of TRI during inhalation exposure was made as a function of the Q_{upt} and measurements of the inhaled dose. In their calculation of exhaled breath concentration, Ramsey and Andersen (1984) assumed that alveolar respiration accounts for 70% of total respiration, with 30% of total respiration delegated to the inhaled air that does not participate in alveolar ventilation. By adding instrumental dead space of the breathing valve in the exposure system in the present study to this assumed physiological dead space, a value of 50% of total respiration was assigned to alveolar ventilation. Therefore, cumulative elimination (Q_{elim}) of TRI was estimated by

$$Q_{elim} = (C_{inh} V_{alv} t) - Q_{upt}, \quad (2)$$

where the alveolar ventilation is $V_{alv} = 0.5\, V_E$ and t is the time interval between sequential sampling of the exhaled breath. As for Q_{upt}, with sequential determination of Q_{elim} it is possible to measure the cumulative elimination of TRI during inhalation exposures. The successive elimination of TRI following exposure was calculated as $Q_{elim} = C_{exh} V_E t$.

The percentage uptake (% Upt) of the total inhaled dose at each successive time point during the inhalation exposure period was calculated as

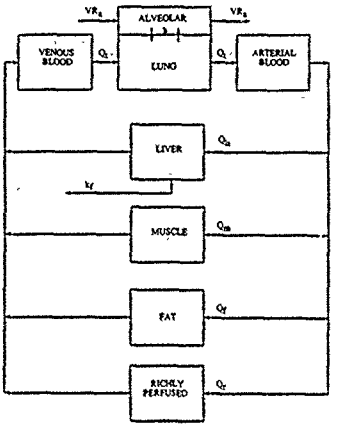

FIG. 2. Diagram of the physiologically based pharmacokinetic model used to simulate the uptake and elimination of inhaled TRI. The symbols and parameters used to describe the model are included in Table 1 and in the equations given under Methods.

$$\%\ \text{Upt} = \frac{(C_{inh} - C_{alv}) \times 100}{C_{inh}}, \quad (3)$$

where the TRI alveolar concentration is $C_{alv} = C_{art}/N$, in which C_{art} is the measured TRI arterial blood level and N is the blood:air partition coefficient for TRI.

A physiologically based pharmacokinetic (PBPK) model was used to describe the disposition of TRI in the rat (Fig. 2). It was assumed that a blood flow-limited model was adequate to characterize the tissue distribution of TRI. Compartmental volumes and organ blood flows were obtained from the literature (Gerlowski and Jain, 1983; Ramsey and Andersen, 1984) and scaled to 340 g, the mean body weight of rats used in the present study. Partition coefficients and the metabolic rate constant for TRI were taken from Gargas *et al.* (1986, 1989), except for the richly perfused tissue blood and lung:blood partition coefficients, which were assumed to be the same as the liver:blood partition coefficient. The lung:air partition coefficient was then derived by multiplying the blood:air coefficient from Gargas *et al.* (1986) by the lung:blood coefficient. The alveolar:lung mass transfer coefficient was estimated from the value used for methylene chloride (Angelo and Pritchard, 1984). Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport

TABLE I

PARAMETERS FOR THE PHYSIOLOGICAL PHARMACOKINETIC MODEL OF TRI IN THE RAT (340 g)

Parameter	Value
Alveolar ventilation rate (ml/min), VR_a	126 (50 ppm exposure) 118 (500 ppm exposure)
Inhaled gas concentration (μg/ml), C_{inh}	0.279 (50 ppm exposure) 2.70 (500 ppm exposure)
Blood flows (ml/min)	
Cardiac output, Q_c	106.4
Fat, Q_f	9.4
Liver, Q_l	39.8
Muscle, Q_m	12.8
Richly perfused, Q_r	44.4
Tissue volumes (ml)	
Blood, V_b	25.4
Fat, V_f	30.5
Liver, V_l	13.6
Muscle, V_m	248.0
Richly perfused, V_r	17.0
Alveolar, V_a	2.0
Lung, V_l	3.97
Partition coefficients	
Lung:air, R_a	8.6
Fat:blood, R_f	47.7
Liver:blood, R_l	1.49
Lung:blood, R_l	1.49
Muscle:blood, R_m	0.55
Richly perfused blood, R_r	1.49
Miscellaneous constants	
Lung:alveolar mass transfer coefficient, k	500 ml/min
Metabolic rate constant, K_l	0.115 min^{-1}

of TRI in the rat were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted TRI concentrations over time. The model-predicted cumulative uptake values were the sum of the simulated amounts of TRI in each tissue compartment in the model.

Analysis of TRI in air and blood. The concentration of TRI in the inhaled and exhaled air samples collected during and following the inhalation exposures were measured with a Tracor MT560 gas chromatograph (GC) (Tracor Instruments, Austin, TX). Analyses for the 500 ppm exposures were conducted using a flame ionization detector (FID), while the analyses for the 50 ppm exposures were conducted using an electron capture detector (ECD). In either case, air samples were procured with a gas-tight, 1-ml syringe and injected directly onto an 8 ft × ¼ in. stainless-steel column packed with 0.1% AT 1000 on GraphPak. Standards were prepared in each of four 9-liter standard bottles with Teflon stoppers containing needles used for taking the air samples with the syringe. Operating temperatures were 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; and 110°C, isothermal column operation. When using the ECD, gas flow rates of 40 ml/min were employed for nitrogen (carrier gas), with an additional makeup gas flow rate of 30 ml/min to the detector.

TRI levels in the blood were measured by GC headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock by a 1-ml syringe. Depending on the anticipated blood concentration, between 25 and 200 μl of the blood was taken from the stopcock with an Eppendorf pipet and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE-lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 autosampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer, Norwalk, CT), where it was heated to 80°C by a high-precision thermostat device. A predetermined volume of the vapor was then injected automatically into the column for analysis. Standard solutions were made and assayed by diluting calculated amounts of pure TRI in toluene, transferring to vials, and analyzing as previously described. The concentration of TRI in the blood samples was then determined from a standard curve generated from blood that was spiked with these standard solutions. The column used was an 8 ft × ¼ in. stainless-steel column packed with FFAP Chromasorb W-AW (80–100 mesh). Operating temperatures were 200°C, injection port; 350°C, ECD detector; and 85°C, column oven. The carrier gas was 5% argon-methane, at a flow rate of 40 ml/min with a makeup gas flow rate of 20 ml/min to the detector.

RESULTS

The target concentrations for the TRI inhalation exposures were 50 and 500 ppm. The starting concentration of TRI in the bag from which the test animal inhaled the test compound was measured just prior to the initiation of each exposure. TRI bag concentrations were 515.8 ± 20.6 and 53.6 ± 2.2 ppm ($\bar{x} \pm$ SE) for the 500 and 50 ppm groups, respectively. The actual concentrations inhaled by the animals were determined by measurements of air samples taken from the airway

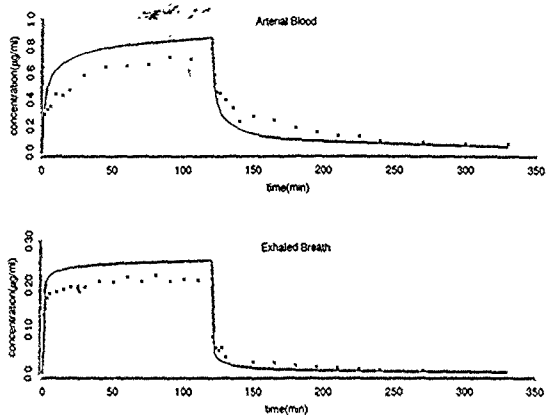

FIG. 3. Observed (●) and model-predicted (—) TRI concentrations in the blood (top graph) and exhaled breath (bottom graph) of rats during and following a 2-hr 50 ppm inhalation exposure. Each point represents the mean value for six rats.

immediately adjacent to the breathing valve. Inhaled TRI concentrations for the six rats in each group were 491.6 ± 11 ppm for the 500 ppm exposures and 51.2 ± 1.2 ppm ($\bar{x} \pm$ SE) for the 50 ppm exposures.

TRI concentrations in the blood and exhaled breath of rats during and following inhalation of TRI are shown for 50 ppm exposures in Fig. 3 and for 500 ppm exposures in Fig. 4. Concentrations of TRI in the exhaled breath generally paralleled concentrations in the arterial blood, though some differences were noted. TRI was rapidly absorbed from the lungs and readily available for distribution to tissues of the body, in that arterial blood concentrations of TRI were quite high at the first sampling time (i.e., 2 min). After an initial rapid rise, the blood levels increased steadily but did not reach steady state by the end of the 2-hr exposures. Exhaled breath levels increased even more rapidly than blood levels after the initiation of exposures, attaining near steady state within 10 to 15 min. The exhaled breath versus time curves were asymptotic, in that they gradually increased throughout the remainder of the 2-hr inhalation period. An increase in the inhaled concentration from 50 to 500 ppm produced an equivalent (i.e., 10-fold) increase in the observed blood and exhaled breath concentrations of TRI. Upon cessation of TRI inhalation, the chemical was rapidly eliminated. As can be seen in Figs. 3 and 4, TRI concentrations in the exhaled breath initially diminished more rapidly than did blood concentrations. Disappearance of TRI from the blood paralleled that in the expired air during the latter part of the postexposure period.

PBPK model-generated blood and exhaled breath concentrations of TRI are shown as solid lines in Figs. 2 and 3. Concentrations of TRI in the expired air were well simulated by the model during and following the 50 and 500 ppm exposures. Model predictions that TRI levels in the exhaled breath would quickly reach near steady state after the exposures began were consistent with the observed data, with the observed levels slightly lower

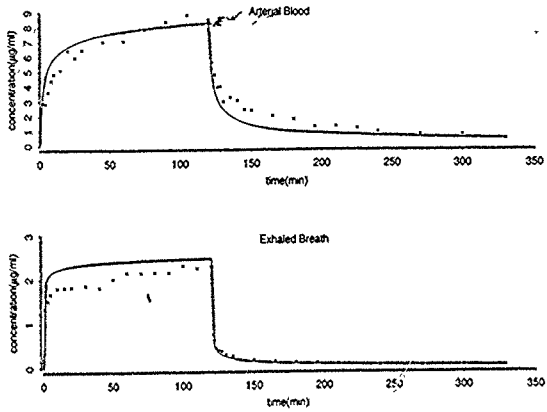

FIG. 4. Observed (●) and model-predicted (—) TRI concentrations in the blood (top graph) and exhaled breath (bottom graph) of rats during and following a 2-hr 500 ppm inhalation exposure. Each point represents the mean value for six rats.

than simulated levels over the course of the 50 and 500 ppm exposures. The model accurately predicted both rapid and slow elimination phases of expiration of TRI postexposure. When the model was used to describe the time course of TRI in the arterial blood, a relatively good fit was obtained during the 500 ppm exposure (Fig. 4). Arterial blood concentrations were overpredicted by approximately 20% during the 50 ppm exposure (Fig. 3). The model predicted a slightly more rapid decline in blood levels postexposure in both groups than was observed during the period of 130–200 min, but levels at subsequent time points were accurately predicted.

Percentage systemic uptake of TRI appeared to be both concentration- and time-dependent (Fig. 5). Although percentage uptake was quite high during the initial minutes of inhalation of 50 and 500 ppm TRI, a decrease of 30–35% occurred during the first hour. Percentage uptake diminished more slowly during the remainder of the exposure period. As can be seen in Fig. 5, the mean values after 10 min are slightly but consistently lower in the 500 ppm group.

Plots of cumulative uptake of TRI during the inhalation sessions, as calculated by Eq.

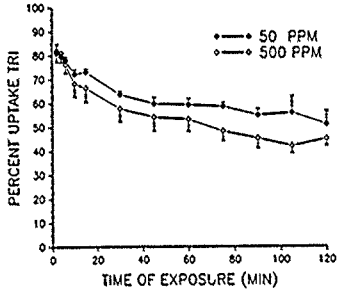

FIG. 5. Percentage uptake of TRI during inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. Each point represents the mean ± SE for six rats. The percentage uptake of the inhaled dose over time was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter.

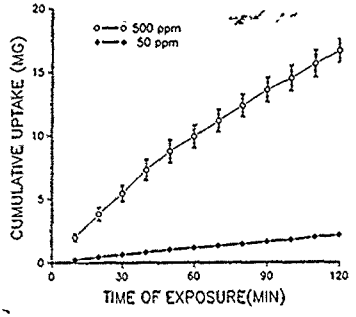

FIG. 6 Cumulative uptake of TRI during inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. The quantity of inhaled TRI retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled TRI concentrations. Each point represents the mean ± SE for six rats. The diminutive SE bars are omitted from the 50 ppm values for sake of clarity.

(1), are shown in Fig. 6. Cumulative uptake, as determined from direct measurements of the minute volume and TRI concentrations in the inhaled and exhaled breath, was not linear in either the 50 or 500 ppm animals. The departure from linearity was more apparent at the higher exposure level. Total cumulative uptake during the 2-hr exposures, as ascertained from the direct measurement data, was 2.2 ± 0.2 and 16.7 ± 0.9 mg ($\bar{x}$ ± SE) in the 50 and 500 ppm groups, respectively. Predicted values for uptake, derived by summating the predicted levels of TRI in the model compartments, were significantly less than these measured uptake values (i.e., after 2 hr exposure to 500 ppm TRI, predicted uptake was 50% of measured uptake).

Cumulative elimination of TRI in the exhaled breath during and following inhalation exposure is shown in Fig. 7. During TRI exposure, TRI in the pulmonary blood and TRI not absorbed from the alveolar space each contribute to the TRI eliminated in the exhaled breath. Cumulative pulmonary elimination, as determined by Eq. (2), was proportional to the inhaled concentration. By the end of the 2-hr exposure to 50 and 500 ppm TRI, 2.1 ± 0.2 and 20.8 ± 3.0 mg ($\bar{x}$ ± SE), respectively, were eliminated from the rats in the exhaled breath. Model-predicted elimination at the end of 2 hr in the 50 and 500 ppm groups was approximately 40 and 50% greater, respectively, than these measured values. Following the termination of exposure, TRI was eliminated in the exhaled breath in progressively smaller quantities, as reflected by postexposure plateaus in the elimination curves. During the 2-hr postexposure period, an additional 0.3 and 3.3 mg of TRI were eliminated from the animals in the 50 and 500 ppm exposure groups, respectively.

DISCUSSION

Pharmacokinetic studies are playing an increasingly important role in toxicology and

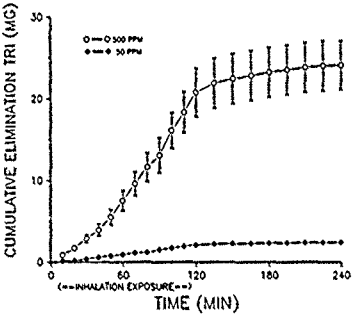

FIG. 7. Cumulative elimination of TRI during and following inhalation exposures. Rats inhaled 50 or 500 ppm TRI for 2 hr. The quantity of inhaled TRI eliminated in the breath over time was calculated using direct measurements of the minute volume and TRI concentrations in the inhaled and exhaled breath. Each point is the mean ± SE for six rats. Cumulative elimination was determined for successive 10-min intervals during the 2-hr exposure, and for successive 15-min intervals postexposure.

in health risk assessments (Clark and Smith, 1984; Clewell and Andersen, 1985; NRC, 1987). Unfortunately, there is relatively little information available on the uptake and disposition of TRI and many other VOCs during ongoing inhalation exposures. Most pharmacokinetic studies of TRI have focused on the elimination of the chemical and its metabolites following the cessation of exposures (Stewart *et al.*, 1969; Ikeda and Ohtsuji, 1972; Eben and Kimmerle, 1974; Seki *et al.*, 1975; Holmberg *et al.*, 1977; Caperos *et al.*, 1982; Schumann *et al.*, 1982a,b). Although Schumann *et al.* (1982a) utilized rats with an indwelling jugular cannula, just three blood samples were taken and analyzed for TRI content during a 6-hr inhalation session. Similarly, blood levels appear to have been taken for TRI analysis only three times from human volunteers during 6 hr of TRI inhalation (Nolan *et al.*, 1984). These few time points are not sufficient to accurately define blood concentration versus time profiles, or to recognize changes which may occur in kinetics during the course of exposures.

The monitoring of blood and breath levels of VOCs in animals has been primarily restricted to times after termination of exposures, due to problems involving restricted access to subjects in inhalation chambers and metabolism cages and technical difficulties in working with small animals. In the present study we have utilized a technique which allowed direct, simultaneous measurements of respiratory parameters, inhaled and exhaled breath concentrations of TRI, and TRI blood levels in unanesthetized rats during exposures. The separation of the inhaled and exhaled breath streams, by use of a miniaturized one-way breathing valve, facilitated accurate serial determinations of airflow and TRI concentrations in the inhaled and exhaled breath. A similar nonrebreathing valve has been used previously for assessing respiratory volumes and gas exchange (Mauderly *et al.*, 1979), though it has apparently not been used in pharmacokinetic studies. This approach allowed us to directly monitor respiratory uptake and elimination of TRI, a VOC for which pulmonary clearance is the major route of elimination. Indeed by the end of the 2-hr 50 and 500 ppm inhalation exposures, we determined that 52.5 and 56.3%, respectively, of the inhaled dose had been exhaled.

Percentage uptake of inhaled TRI was highly time-dependent. The percentage uptake of inhaled TRI has apparently not been previously determined in laboratory animals. Schumann *et al.* (1982a) assumed that 60% of inspired TRI was absorbed by rats throughout a 6-hr exposure. The rate of transfer of TRI from alveoli to blood should initially be very rapid, but become progressively slower as the chemical accumulates in the blood and tissues. This pattern was reflected by the time course of systemic uptake of TRI in the current study, where percentage uptake decreased from more than 80% at the beginning to less than 50% at the end of the 2-hr exposure. Initial uptake of inhaled TRI is governed by tissue loading and metabolism. Once the tissues have reached steady state, continued uptake will be dependent upon the rate of metabolism of the chemicals. Since TRI is very poorly metabolized by the rat and by humans (Ikeda and Ohtsuji, 1972; Schumann *et al.*, 1982a; Nolan *et al.*, 1984), percentage uptake would be expected to be very low once steady state was reached. Steady state was not reached in our study, as percentage uptake progressively decreased over the 2 hr of exposure. Monster *et al.* (1979) found that percentage uptake of inhaled TRI by humans decreased rapidly from approximately 95% at the onset to 30% at the end of 4-hr exposures. Nolan *et al.* (1984) reported that human volunteers exposed for 6 hr to 35 or 350 ppm TRI retained about 25% of the chemical to which they were exposed. The greater percentage uptake in rats than in humans is consistent with a higher TRI blood:air partition coefficient and greater cardiac output/pulmonary blood flow in rats

than in humans (Reitz *et al.*, 1988). Since systemic uptake of TRI is time-dependent, average values of percentage uptake for short intervals may be misleading and have little relevance for health risk assessments.

TRI exhibits linear kinetics over a wide dosage range. Exhaled breath levels and blood levels of TRI were directly proportional to the inhaled concentration (i.e., 50 and 500 ppm) of TRI throughout the 2-hr exposures in the current study. Similar findings were reported in humans exposed for 6 hr to 35 and 350 ppm TRI (Nolan *et al.*, 1984). Schumann *et al.* (1982a) found that the amount of TRI exhaled by rats and mice increased eight- to ninefold when the inhaled concentration of TRI was increased from 150 to 1500 ppm. These investigators also observed that blood levels, tissue levels, and body burden of ^{14}C-TRI were each proportional to exposure level in both species. Although TRI was poorly metabolized, Schumann *et al.* (1982a) demonstrated that its biotransformation by mice and rats was a dose-dependent, saturable process. Metabolic saturation in rats was believed to occur between 500 and 1500 ppm, if not near 500 ppm. Metabolic saturation, however, had little apparent effect on the overall pharmacokinetics of TRI, since biotransformation was a minor route of elimination. The kinetics of TRI is governed largely by its partition coefficients (e.g., blood:air, tissue:blood) and the physiology (e.g., respiratory rate and volume, cardiac output, tissue volumes, and blood flow rates) of the animal.

The major route of elimination of TRI in laboratory animals and in man is exhalation of the parent compound (Schumann *et al.*, 1982a; Nolan *et al.*, 1984). Nolan and his colleagues measured TRI in the exhaled breath of male human volunteers during and after 6-hr inhalation sessions. The exhaled breath levels after 1.5 hr of exposure to 35 and 350 ppm TRI were 0.14 and 1.28 μg/ml, respectively. Assuming a linear scaleup to a 50 and 500 ppm exposure, the exhaled breath levels in humans would be 0.2 and 1.33 μg/mL. These values are quite comparable to exhaled breath levels measured in the present study after 1.5 hr of exposure of rats to 50 and 500 ppm TRI (i.e., 0.21 and 2.16 μg/mL, respectively). The similarity in magnitude in exhaled breath levels of TRI between rats and man is an unexpected finding. It would be anticipated that alveolar and presumably exhaled breath concentrations of TRI would be lower in rats than in man, due to the rat's higher blood:air partition coefficient and greater percentage uptake of inhaled TRI.

The aforementioned physiological parameters and biochemical constants were used to input into a PBPK model for inhalation of TRI. Our model accurately predicted the time courses of TRI in the blood and exhaled breath of rats both during and following exposure to 50 and 500 ppm TRI. Cumulative uptake over the 2-hr exposure, however, was underpredicted by our model. The source of the discrepancy between the predicted and the measured uptake value is unclear. Revisions of the model may be warranted by findings in ongoing studies of TRI concentrations in tissues of exposed animals. These data should be useful in verifying tissue:blood partition coefficients, tissue compartments, and tissue volumes. Reitz *et al.* (1988), for example, found that most of the changes in the pharmacokinetics of TRI in older rats could be accounted for by increasing the size of the fat compartment in their PBPK model. Reitz *et al.* (1988) used their model to accurately predict blood and exhaled breath concentrations measured in humans subjected to TRI inhalation exposures. The investigators also utilized the PBPK model to predict TRI blood levels and amounts metabolized post-exposure in mice, rats, and humans, as well as to describe the kinetics of TRI in rats after iv injection, bolus gavage, and drinking water administration. Thus, it appears that PBPK models can be quite useful in predicting the time course of TRI concentrations in the

body of different species under different exposure conditions.

Major species differences have been observed in the pharmacokinetics of inhaled TRI. After 1.5 hr of exposure to 35 or 350 ppm TRI, humans had mean blood levels of 0.14 and 1.62 μg/ml, respectively (Nolan *et al.*, 1984). After being normalized for differences in inhaled concentrations, mean blood levels in rats in the present investigation were approximately 3.6-fold higher than the levels measured in humans. Schumann *et al.* (1982a) reported blood levels of TRI in rats similar to those observed in the present study (when normalized for inhaled concentration). The lower blood levels in humans are consistent with a lower TRI blood:air partition coefficient for man (2.53 versus 5.76 for rats) and the greater adipose tissue volume in man (23.1% versus 11% in rats). In a comparison with the normalized data of Schumann *et al.* (1982a), Nolan *et al.* (1984) noted that blood levels in mice and rats inhaling TRI were 17.3 and 3.5 times higher, respectively, than those measured in humans. When determining the actual inhaled dose at equivalent inhaled concentrations, one must consider the wide variation in volume of respiration and body weight between species. Assuming a 4.2 liters/min alveolar ventilation and 70 kg body wt for man (Ganong, 1979), the rats in the present study received an inhaled dose approximately six times greater than that of the humans in the study by Nolan *et al.* (1984). Nolan and his colleagues determined following a 6-hr exposure to 150 ppm TRI that mice, rats, and humans metabolized 0.16, 0.06, and 0.014 μmol/kg/ppm, respectively. Thus, mice and rats should be more susceptible than humans to TRI toxicity at equivalent inhaled concentrations, due to significantly greater systemic absorption and metabolism of the chemical.

Meaningful health risk assessments require a careful selection of the measure of dose. In the present study systemic uptake of TRI is measured directly during the initial phase of inhalation exposure, when significant loading of tissues is occurring. Once tissue loading is completed (i.e., steady state is reached), very little uptake of TRI should occur because of the poor metabolism of the chemical. Thus, the common practice of assessing dose by multiplying ventilation rate by inhaled concentration would be very misleading during prolonged exposures. A more logical measure of target organ dose or tissue exposure would be the area under the tissue concentration versus time curve. The concept and rationale for selection of appropriate target organ dose measures (i.e., tissue dosimetry) are discussed in detail by Andersen (1987). It is important that target organs and mechanisms of toxicity be elucidated, so that the agent(s) responsible for toxic effects are identified and can subsequently be quantified and correlated with the magnitude of toxicity in the target tissue(s). It is not clear for TRI whether the parent compound or its metabolites should serve as the dose measure, or surrogate. Near-lethal exposures are required for effects on most target organs. Carcinogenicity bioassays have been negative, or inconclusive. Reitz *et al.* (1988) decided to use the average concentration of TRI in the liver over a lifetime (ACL) as a dose surrogate. These investigators used a PBPK model to calculate ACLs for comparison of internal doses received by mice and rats in long-term toxicity studies versus humans drinking TRI-contaminated water. Unfortunately, there is a paucity of data on actual concentrations of TRI in the liver and other organs. Direct measurement studies are needed to generate tissue concentration versus time data sets for rigorous validation of PBPK model predictions of dose surrogates.

APPENDIX

*Mass Balance Differential Equations for Physiological Pharmacokinetic Model**

Arterial Blood

$$V_b \frac{dC_b}{dt} = Q_l\left(\frac{C_l}{R_l} - C_b\right).$$

Venous Blood

$$V_b\frac{dC_v}{dt} = Q_{li}\frac{C_{li}}{R_{li}} + Q_m\frac{C_m}{R_m} + Q_f\frac{C_f}{R_f} + Q_r\frac{C_r}{R_r} - Q_tC_v.$$

Alveolar Space

$$V_a\frac{dC_a}{dt} = VR_aC_{inh}u_1(t)^{**} - VR_aC_a + h\left(\frac{C_l}{R_a} - C_a\right).$$

Lung

$$V_l\frac{dC_l}{dt} = Q_t\left(C_v - \frac{C_l}{R_l}\right) + h\left(C_a - \frac{C_l}{R_a}\right).$$

Liver

$$V_{li}\frac{dC_{li}}{dt} = Q_{li}\left(C_b - \frac{C_{li}}{R_{li}}\right) - k_f\frac{X_{li}}{R_{li}}.$$

Muscle

$$V_m\frac{dC_m}{dt} = Q_m\left(C_b - \frac{C_m}{R_m}\right).$$

Fat

$$V_f\frac{dC_f}{dt} = Q_f\left(C_b - \frac{C_f}{R_f}\right).$$

Richly Perfused

$$V_r\frac{dC_r}{dt} = Q_r\left(C_b - \frac{C_r}{R_r}\right).$$

Note. $^*C_i$ = concentration of TRI in compartment i. See Table 1 for the definition of the other symbols. $^{**}\mu(t) = 1$ for $t \leq 120$ min and 0 for $t > 120$ min.

ACKNOWLEDGMENTS

The authors are grateful to Ms. Linda Quigley, Miss Elizabeth Lehman, and Mrs. Judy Bates for their expertise in preparation of this manuscript.

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APPENDIX B

GALLEY PROOF OF PAPER IN PRESS IN
TOXICOLOGY AND APPLIED PHARMACOLOGY

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats." In press, Toxicology and Applied Pharmacology, 1991.

TOXICOLOGY AND APPLIED PHARMACOLOGY 110, 000–000 (1991)

Physiological Pharmacokinetic Modeling of Inhaled Trichloroethylene in Rats[1,2]

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Received October 15, 1990; accepted June 1, 1991

Physiological Pharmacokinetic Modeling of Inhaled Trichloroethylene in Rats. DALLAS, C. E., GALLO, J. M., RAMANATHAN, R., MURALIDHARA, S., AND BRUCKNER, J. V. (1991). *Toxicol. Appl. Pharmacol.* 110; ■■■-■■■. The pharmacokinetics of trichloroethylene (TCE) was characterized during and following inhalation exposures of male Sprague-Dawley rats. The blood and exhaled breath TCE time-course data were used to formulate and assess the accuracy of predictions of a physiologically based pharmacokinetic (PB-PK) model for TCE inhalation. Fifty or 500 ppm of TCE was inhaled by unanesthetized rats of 325–375 g for 2 hr through a miniaturized one-way breathing valve. Repetitive samples of the inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently during and for 3 hr following the exposures and analyzed for TCE by headspace gas chromatography. Respiratory rates and volumes were continuously monitored and used in conjunction with the pharmacokinetic data to delineate uptake and elimination profiles. Levels of TCE in the exhaled breath attained near steady-state soon after the beginning of exposures, and were then directly proportional to the inhaled concentration. Exhaled breath levels of TCE in rats were similar in magnitude to values previously published for TCE inhalation exposures of humans. Levels of TCE in the blood of the 50 ppm-exposed animals also rapidly approached near steady-state, but blood levels in the 500 ppm-exposed animals rose progressively, reaching concentrations 25- to 30-fold higher than in the 50 ppm group during the second hour of exposure. The 10-fold increase in inhaled concentration resulted in an 8.7-fold increase in cumulative uptake, or total absorbed dose. These findings of nonlinearity indicate that metabolic saturation ensued during the 500 ppm exposure. The PB-PK model was characterized as blood flow-limited with TCE eliminated unchanged in the exhaled breath and by saturable liver metabolism. The uptake and elimination profiles were accurately simulated by the PB-PK model for both the 50 and 500 ppm TCE exposure levels. Such a model may be quite useful in risk assessments in predicting internal (i.e., systemically absorbed) doses of TCE and other volatile organics under a variety of exposure scenarios. © 1991 Academic Press, Inc.

Trichloroethylene (1,1,2-trichloroethylene, TCE) is a volatile organic compound (VOC) which has been widely used as a surgical anesthetic, fumigant, extractant in food processing, metal degreaser, dry cleaning agent, and solvent in other commercial applications. It has been estimated that of 3.5 million persons believed to be occupationally exposed to TCE in the U.S., at least 100,000 workers are exposed full-time, and that two-thirds of these

[1] This research was sponsored by U.S. EPA Cooperative Agreements CR 812267 and CR 816258 and by the Air Force Office of Scientific Research, Air Force Systems Command, USAF, under Grant AFOSR 87-0248. The U.S. Government's right to retain a nonexclusive royalty-free license in and to the copyright covering this paper, for governmental purposes, is acknowledged.

[2] Presented in part at the 26th Annual Meeting of the Society of Toxicology, Washington, D.C., February, 1987.

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are in work environments where there are not adequate control measures (NIOSH, 1978). Although excessive exposures to TCE vapors have resulted in cardiac arrhythmias and in central nervous system depression, most occupational exposures do not result in apparent manifestations of toxicity (Defalque, 1965; NIOSH, 1973; U S. EPA, 1985). There is considerable concern, however, that TCE may be a human carcinogen. TCE has been reported to produce an increased incidence of hepatocellular carcinoma in B6C3F1 mice subjected daily for their lifetime to high oral doses of the chemical (NCI, 1976; NTP, 1983). More recent studies have also shown that TCE can be carcinogenic in animals upon inhalation exposure (Fukuda *et al.*, 1983; Maltoni *et al.*, 1988).

Assessment of toxic and carcinogenic risks of exposure to TCE and other VOCs has become a subject of major importance over the last decade. Although it has formerly been common practice to calculate risk estimates on the basis of administered dose-toxicity/tumor incidence data, it is now recognized that the internal, or target organ dose is a more accurate and direct determinant of the magnitude of injury. The dose of chemical actually reaching a target organ is dependent upon kinetic processes which may vary considerably with the administered dose, route of exposure, and animal species. Thus, recognition and use of pharmacokinetic data can substantially reduce uncertainties inherent in the route-to-route, high-dose to low-dose and species-to-species extrapolations often necessary in risk assessment (Gehring *et al.*, 1976; Clewell and Andersen, 1985; NRC, 1987).

There have been a relatively large number of studies of the pharmacokinetics of TCE in humans, but data on the time-course of alveolar and blood levels during ongoing inhalation exposures are quite limited. Most human studies have focused on the elimination of TCE and its major metabolites postexposure (Stewart *et al.*, 1970; Kimmerle and Eben, 1973a; Monster *et al.*, 1976; Sato *et al.*, 1977). Additional studies to obtain TCE time-course profiles are increasingly limited by the ethical question of exposing persons to a potential human carcinogen. Thus, investigations utilizing laboratory animals must be largely relied on to provide such information.

Surprisingly, there are relatively few data available in the literature on the time-course of TCE or its metabolites in laboratory animals inhaling the chemical. Most existing studies are limited to the elimination phase following exposure (Kimmerle and Eben, 1973b; Nakajima *et al.*, 1988; Fisher *et al.*, 1989). Technical difficulties with measuring solvent uptake and respiratory functions serially in small animals during inhalation exposures have hindered accurate definition of TCE uptake and elimination profiles. Prout *et al.* (1985) did investigate the time-course of TCE and its major metabolites in the bloodstream of mice and rats given a 1,000 mg/kg oral dose of TCE in corn oil. The study results are useful qualitatively in that they reveal that TCE undergoes much more extensive first-pass metabolism in the mouse than in the rat. The results are of limited use quantitatively, however, in that blood was collected from only one animal at each time-point. Balance studies in mice and rats administered [^{14}C]TCE orally (Prout *et al.*, 1985; Dekant *et al.*, 1986) and by inhalation (Stott *et al.*, 1982) confirm that mice have a higher TCE metabolic capacity than do rats. In each study, ^{14}C levels in animal tissues were measured only at a single time (i.e., 50 or 72 hr) postexposure. Thus, blood and tissue TCE concentration versus time data that are presently available are not adequate to delineate the internal dose of TCE received during inhalation exposures.

Physiologically based pharmacokinetic (PB-PK) models have been formulated for a number of VOCs, in an effort to better understand and forecast the dynamics of the chemicals in the blood and tissues of laboratory animals and humans. The NRC (1986) was the first to describe the use of a PB-PK model for TCE in route-to-route and rat-to-human extrapolations. Bogen (1988) applied the styrene PB-PK model of Ramsey and Andersen (1984) to

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predict relationships between the administered dose of TCE, the toxicologically effective dose, and the risk of cancer in humans. Experimental data were not supplied in either case, however, to test the fidelity of the TCE model predictions. Fisher *et al.* (1989) recently developed a PB-PK model to describe the dynamics of TCE and trichloroacetic acid in pregnant rats exposed to TCE by inhalation and ingestion. The model simulations compared favorably with the limited blood TCE concentration time data which were available to the investigators.

In consideration of the foregoing, the objectives of the present investigation were to: (a) quantify the rate and magnitude of TCE uptake and elimination over time during the course of TCE inhalation exposures of rats, (b) accurately define blood and exhaled breath TCE concentration versus time profiles during and after the exposures, and (c) formulate a PB-PK model for inhalation of TCE based on the observed time-course data.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light–dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. There were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided *ad libitum*. The rats were used after at least a 14-day acclimation period, at which time their body weight ranged from 325–375 g. TCE exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test material. Trichloroethylene (TCE), of >99.99% purity, was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). The purity of the solvent was verified by gas chromatography.

Animal preparation. All rats were surgically implanted with an indwelling carotid artery cannula, which exited the animal at the back of the neck. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml), acepromazine maleate (10 mg/ml), xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v:v:v). The cannulated animals were maintained in a harness and pulley system that allowed relative freedom of movement in metabolism cages during a 24-hr recovery period after surgery.

Inhalation exposures. A known concentration of TCE was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of TCE into the bag filled with air. Uniform dispersion of the vapor was insured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, a miniaturized one-way breathing valve (Hans Rudolph, Inc., St. Louis, MO), and an empty 70-liter gas collection bag. The latter bag served as a reservoir to collect exhaled gas. The breathing valve was attached to a face mask designed to fit the rat, so that the valve entry port was directly adjacent to the nares of the test animal. Thus, separate and distinct airways for the inhaled and exhaled breath streams were established. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneva, Switzerland). The face mask was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. This basic inhalation exposure setup has been illustrated previously by Dallas *et al.* (1989). TCE exposures were initiated only after stable breathing patterns were established during a 1-hr acclimation period. During the 2 hr of TCE exposure and for 3 hr afterward, serial inhaled and exhaled breath samples were taken at approximately the same time as blood samples from the carotid artery cannula. The blood samples were then analyzed for TCE content by headspace gas chromatography, whereas the air samples were injected directly into the gas chromatograph.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored according to previously published methods (Dallas *et al.*, 1983, 1986). The airflow created by the animal's inspiration was recorded both during and following TCE inhalation exposure in terms of minute volume (volume of respiration per minute, or V_E), respiratory rate (f), and tidal volume (V_T). Mean values for these parameters were obtained by averaging the measurements taken at 10-min intervals in individual animals during the 2-hr exposures. Mean ± SE values for the 500 ppm-exposure group ($n = 6$) were: $V_E = 218.0 \pm 20.2$ ml/min, $f = 128.4 \pm 7.1$ breaths/min, and $V_T = 1.71 \pm 0.15$ ml. Means ± SE for the 50 ppm group ($n = 6$) were: $V_E = 268.9 \pm 15.5$ ml/min, $f = 132.0 \pm 7.3$ breaths/min, and $V_T = 2.12 \pm 0.20$ ml.

Calculations of TCE uptake and elimination were conducted utilizing the equations presented in a previous VOC inhalation study in rats (Dallas *et al.*, 1989). Since the V_E and the exhaled breath TCE concentration at each sampling point were measured, subtraction of the quantity of TCE exhaled by the animal from the amount inhaled yielded an estimation of the quantity of TCE taken up during sequential sampling periods (cumulative uptake). The percentage uptake during each exposure period was determined by dividing the cumulative uptake by the total inhaled dose for that time period. The statistical signifi-

cance of differences between the 50 and 500 ppm animals in percentage uptake at each time point was assessed by paired and unpaired t tests, with $p < 0.05$ chosen as the minimum level of significance.

PB-PK model. A PB-PK model was used to describe the disposition of TCE in the rat (Fig. 1). It was assumed that a blood-flow-limited model was adequate to characterize the tissue distribution of TCE. Previous PB-PK models for VOCs have utilized blood-flow-limited organ representations (Ramsey and Andersen, 1984; Angelo and Pritchard, 1984; Dallas *et al.*, 1989). Compartmental volumes and organ blood flows were obtained from the values used by Ramsey and Andersen (1984) for rats, and scaled to 340 g, the mean body weight of rats utilized in the present study. Tissue blood partition coefficients that characterize the extent of tissue TCE uptake were obtained from Andersen *et al.* (1987). The Michelis-Menton parameters, V_{max} and K_m, describing the rate of TCE metabolism, were initially estimated from Andersen *et al.* (1987), and were $K_m = 0.25$ μg/ml and $V_{max} = 183.3$ μg/kg/min. When scaled to the 340-g rat used in the current study, $V_{max} = 82.0$ μg/min. The final value of V_{max} set equal to 75.0 μg/min, provided good agreement between observed and predicted blood TCE concentrations. Differential mass balance equations, incorporating the parameters listed in Table 1, that describe the transport of TCE in the rat as depicted in Fig. 1, were numerically solved with the ACSL (Advanced Continuous Simulation Language) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted blood and exhaled breath TCE concentrations as a function of time.

Analysis of TCE in air and blood. The concentration of TCE in the inhaled and exhaled air was measured with a Tracor MT560 gas chromatograph (Tracor Instruments, Austin, TX). Standards were prepared in each of four 9-liter bottles equipped with Teflon stoppers with needles from which air samples could be taken by syringe. Analyses for the 500 ppm exposures were conducted using a flame ionization detector (FID), while the analyses for the 50 ppm inhalation exposures were conducted using an electron capture detector (ECD). The detection limits for TCE in air by FID and ECD were 0.5 and 0.003 μg/ml, respectively. The ECD detector was employed for the 50 ppm exposures, since most of the postexposure exhaled breath concentrations in these animals were below the FID detection limit. In either case, air samples were procured with a gas-tight, 1-ml syringe and injected directly onto

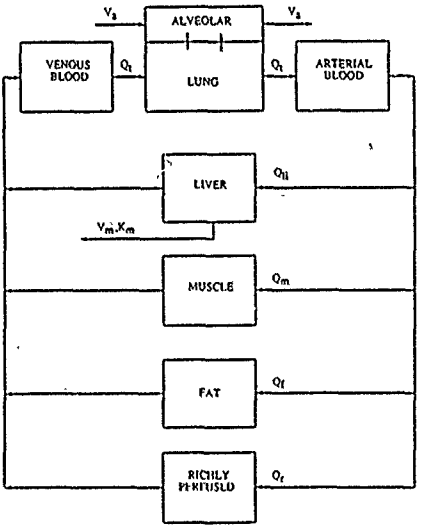

FIG. 1. Diagram of the physiological pharmacokinetic model used to simulate the uptake and elimination of inhaled TCE. The symbols and parameters used to describe the model are included in Table 1.

TABLE 1

PARAMETERS FOR THE PHYSIOLOGICAL PHARMACOKINETIC MODEL OF INHALED TCE IN THE RAT (340 g)

Parameter	Value
Alveolar ventilation rate (ml/min), VR_a	134.5 (50 ppm exposure) 109 (500 ppm exposure)
Inhaled gas concentration (μg/ml)	0.272 (50 ppm exposure) 2.69 (500 ppm exposure)
Alveolar mass transfer coefficient	500 ml/min
Blood flows (ml/min)	
Cardiac output, Q_c	106.4
Fat, Q_f	9.4
Liver, Q_l	39.8
Muscle, Q_m	12.8
Richly Perfused, Q_r	44.4
Tissue volumes (ml)	
Alveolar	2.0
Blood	25.4
Fat	30.5
Liver	13.6
Lung	3.97
Muscle	248.0
Richly Perfused	15.2
Partition coefficients	
Blood:Air	21.9
Fat:Blood	25.3
Liver:Blood	1.24
Muscle Blood	0.46
Richly Perfused:Blood	1.24
Metabolism Constants	
V_{max} (μg/min)	75.0
K_m (μg/ml)	0.25

an 8-ft × ¼-in. stainless-steel column packed with 0.1% AT 1000 on GraphPak. Operating temperatures were 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; and 110°C, isothermal column operation. When using the ECD, gas flow rates of 40 ml/min were employed for nitrogen (carrier gas), with an additional make-up gas flow rate to the detector of 30 ml/min.

TCE levels in the blood were measured by gas chromatographic headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock into a 1-ml syringe. Depending on the anticipated blood TCE concentration, from 25 to 200 μl of the blood was taken from the stopcock with an Eppendorf pipet and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 auto-sampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer, Norwalk, CT), where it was heated to a preset temperature by a high precision thermostat device. A predetermined volume of the vapor was then injected automatically into the column for analysis. Standard curves were generated on each day that measurements were conducted by injection of known quantities of TCE into headspace vials for subsequent analysis. The column used was an 8-ft × ¼-in. stainless-steel column packed with FFAP chromasorb W-AW (80–100 mesh). Operating temperatures were 250°C, injection port; 350°C, ECD detector; and 80°C column oven. The carrier gas was 5% argon–methane, at a flow rate of 40 ml/min, with a make-up gas flow rate to the detector of 20 ml/min.

RESULTS

While 50 and 500 ppm were the target TCE inhalation concentrations, the actual concentrations inhaled by the animals were determined by analysis of air samples taken from the airway immediately adjacent to the breathing valve. Mean (±SE) inhaled TCE concentrations for the six rats in each group were 499.8 ± 12.7 ppm for the 500 ppm exposures and 50.7 ± 0.8 ppm for the 50 ppm exposures.

The time-courses of TCE concentrations in the exhaled breath and arterial blood were delineated during and for 3 hr following 2-hr exposures of rats to 50 (Fig. 2) and 500 ppm (Fig. 3) TCE. TCE was readily absorbed from the lungs into the arterial circulation, as reflected by relatively high blood levels at the initial sampling time (i.e., 1 min). The concentration of TCE increased rapidly in the blood of the 50 ppm animals, reaching near steady-state levels within approximately 25 min. In contrast, blood levels in the 500 ppm animals increased steadily, but did not reach steady-state after 2 hr of TCE inhalation. The arterial concentrations were not proportional to the inhaled concentrations. Blood levels during the latter hour of exposure were 25–30 times higher in the 500 than in the 50 ppm group. Exhaled breath levels of TCE increased more rapidly than did blood levels after the initiation of exposures, the former attaining near steady-state within 10–15 min. The exhaled breath TCE concentrations remained

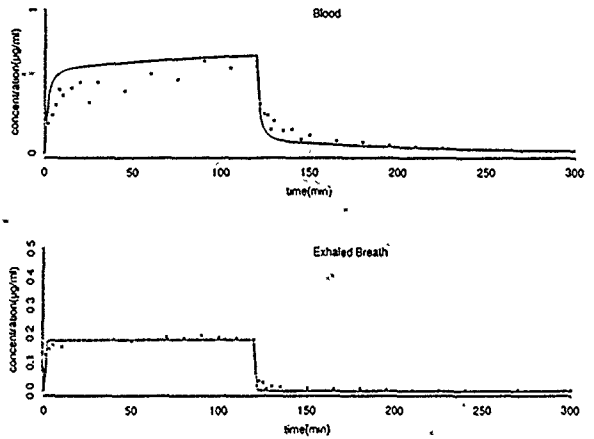

FIG. 2. Observed (●) and model-predicted (—) TCE concentrations in the arterial blood and exhaled breath of rats during and following a 2-hr, 50 ppm inhalation exposure. Each point represents the mean value for six rats.

relatively constant for the 2-hr duration in both exposure groups. Unlike blood levels, the exhaled breath levels at near steady-state were directly proportional to the inhaled concentrations. Mean TCE concentrations in the expired air during the last 1.5 hr of the 2-hr exposures were 34.6 ± 1.1 and 340.8 ± 10.6 ppm ($\bar{x} \pm SE$, $n = 6$) in the 50 and 500 ppm groups, respectively.

The disappearance of TCE from the blood generally paralleled that in the expired air postexposure, though some disparity was observed. Concentrations of TCE measured in the exhaled breath and blood initially decreased very rapidly after exposures ceased. As can be seen in Figs. 2 and 3, the patterns of elimination differed, in that blood levels diminished more slowly than exhaled breath levels during the first 30 to 45 min postexposure. This difference was most pronounced in the 500 ppm group. Thereafter, TCE was eliminated from the blood and breath at comparable rates. The TCE levels were not monitored long enough postexposure to accurately define the terminal elimination half-lives.

The PB-PK model accurately described the uptake and elimination of TCE in both the blood and expired air. Model-generated exhaled breath and blood TCE concentrations are represented by solid lines in Figs. 2 and 3. The predictions of exhaled breath levels during inhalation were in close agreement with the direct measurements of expired TCE at both exposure levels. Postexposure exhaled breath concentrations were well simulated for the 50 ppm group, and only slightly underpredicted during the first 45 min for the 500 ppm group. The progressive increase in blood concentration during the 2-hr, 500 ppm exposure was accurately forecast by the model. The pattern of uptake of TCE into the blood of the 50 ppm animals was adequately described, although the TCE concentrations were slightly overpredicted (i.e., by about 0.1 μg/ml). The model predicted a more rapid postexposure decline in blood levels than was observed during the

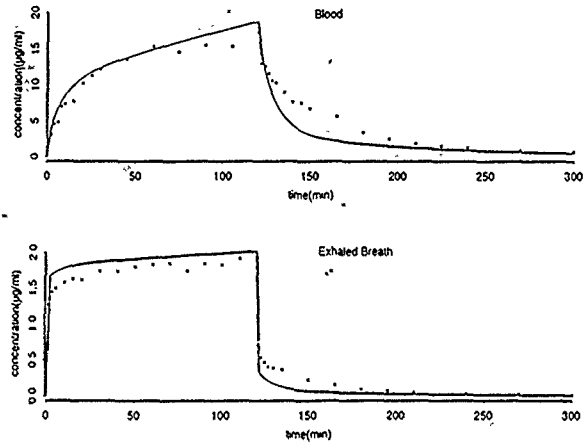

FIG. 3. Observed (●) and model-predicted (—) TCE concentrations in the arterial blood following a 2-hr, 500 ppm inhalation exposure. Each point represents the mean value for six rats.

first hour in the 500 ppm group. Predicted and observed postexposure blood concentrations compared favorably, however, for the 50 ppm rats.

Percentage systemic uptake of TCE was time- but not concentration-dependent (Fig. 4). Uptake exceeded 90% during the first 5 min in both exposure groups, but decreased rapidly over the next 30 min. Thereafter, there was a relatively slow decline in uptake for the remainder of the 2-hr exposure. Percentage uptake appeared to be somewhat higher in the 50 than the 500 ppm animals during much of the first hour, though at no time point was there a statistically significant difference. Percentage systemic uptake values ($\bar{x} \pm$ SE, $n = 6$) during the second hour of exposure for the 50 and 500 ppm groups were 69.9 ± 0.5% and 71.1 ± 0.8%, respectively. There was a total cumulative uptake ($\bar{x} \pm$ SE) during the 2-hr period of 2.96 ± 0.32 mg, or 8.4 mg/kg, in the 50 ppm animals and 24.3 ± 1.2 mg, or 73.3 mg/kg, in the 500 ppm animals (Fig. 5)

DISCUSSION

There is a lack of definitive information on the systemic uptake and disposition of inhaled TCE during exposures, largely due to technical difficulties in accurately monitoring TCE lev-

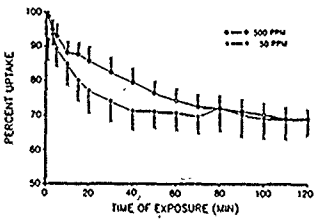

FIG. 4. Percentage systemic uptake of TCE over time during inhalation exposures. Rats inhaled 50 or 500 ppm TCE for 2 hr. Each point represents the mean ± SE for six rats. Percentage uptake of the inhaled dose was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter.

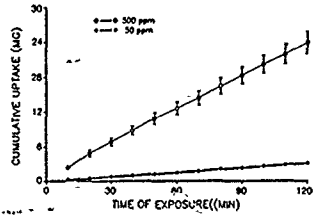

FIG. 5. Cumulative uptake of TCE during inhalation exposures. Rats inhaled 50 or 500 ppm TCE for 2 hr. The quantity of inhaled TCE retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled TCE concentrations. Each point represents the mean ± SE for six rats.

els in the blood and breath of laboratory animals and humans. It might be anticipated that TCE would behave similarly to other relatively water-insoluble gases. This proved to be the case in the current study. As TCE is a small, uncharged, lipophilic molecule, it is readily absorbed across membranes of the pulmonary capillary bed into the systemic circulation. The net rate of transfer from alveoli to blood is initially very rapid, but becomes progressively slower as the chemical accumulates in the blood and tissues. The approach to equilibrium in the blood and exhaled breath is quite rapid, indicative of TCE's relatively low solubility in blood and the slow perfusion of adipose tissue, the major site of deposition of the chemical. These processes are reflected by the time-course of systemic uptake of TCE, where percentage uptake decreases over time from ≥95% at the beginning of exposures to relatively constant levels of 69–71% at near steady-state. Studies in humans reveal lower uptake of inhaled TCE, with values ranging from 44 to 58% (Bartonicek, 1962; Astrand and Ovrum, 1976; Monster *et al.*, 1976, 1979). The greater percentage uptake in rats can be attributed in part to a difference in blood:air partition coefficients, in that values for the rat are 2½ to 3 times higher than for humans (Sato *et al.*, 1977; Gargas *et al.*, 1989). Other contributing factors to the species difference likely include the higher respiratory rate and cardiac output of the rat, as well as its greater apparent capacity to metabolize TCE.

Sequential measurements of TCE uptake during the 2-hr inhalation sessions made it possible to accurately monitor the cumulative uptake (i.e., quantity retained in the body, or absorbed dose) of the chemical. There was a cumulative uptake of 8.4 mg/kg in rats inhaling 50 ppm TCE for 2 hr. When adjusted for exposure concentration and duration, this value is about four times the cumulative uptake reported by Monster *et al.* (1979) in humans inhaling 70 ppm TCE for 4 hr. Thus, rats received a substantially greater systemic dose of TCE on a mg/kg bw basis than do humans at equivalent inhaled concentrations.

Findings in the current study indicate that the rat's capacity to assimilate and metabolize TCE is exceeded during the course of the 2-hr, 500 ppm exposure. Although exhaled breath levels of TCE were directly proportional to the inhaled concentration, arterial blood levels rose 25- to 30-fold with the 10-fold increase in exposure. The blood levels of the 500 ppm animals progressively increased over the 2-hr period, rather than approaching equilibrium as was the case at 50 ppm. Stott *et al.* (1982) saw evidence of metabolic saturation in rats exposed for 6 hr to 600 ppm [^{14}C]TCE. Metabolic saturation was manifest by a decrease in metabolism and increase in exhalation of TCE, when the exposure level was increased from 10 to 600 ppm. Filser and Bolt (1979) calculated the saturation point for TCE metabolism to be 65 ppm on the basis of indirect vapor uptake studies in male rats. Andersen *et al.* (1987), also utilizing data from gas uptake experiments in male rats, determined the V_{max} for TCE to be 11 mg/kg/hr. In the present study, the 10-fold increase in inhaled concentration (i.e., 50 to 500 ppm) resulted in an 8.7-fold increase in cumulative uptake, or total absorbed dose. Thus, metabolic saturation apparently commenced during the course of exposure, when there had

been systemic uptake of a finite quantity of TCE.

The capacity to metabolize TCE has been demonstrated by other investigators to be species-dependent. The metabolic saturation observed by Stott *et al.* (1982) in rats inhaling 600 ppm TCE for 6 hr was not seen at this exposure level in mice. The dose of TCE required to produce metabolic saturation in humans has not been clearly defined. Astrand and Ovrum (1976) saw no evidence of metabolic saturation in men inhaling 100 or 200 ppm TCE for up to 2 hr, in that percentage uptake was constant and absorbed dose was directly proportional to the inhaled concentration. Ikeda *et al.* (1972) reported that urinary concentrations of total trichloro compounds and trichloroethanol in occupationally exposed workers were proportional to inhaled concentrations of up to 175 ppm, but that there was a relative decrease in trichloroacetic acid at exposures above 50 ppm.

The major routes of elimination of TCE are metabolism and exhalation of the parent compound. The elimination of TCE in the exhaled breath generally paralleled elimination of the chemical from the bloodstream of rats in the present investigation. This pattern of elimination of TCE in the blood and breath is also typically seen in humans, although clearance is prolonged (Sato *et al.*, 1977; Nomiyama and Nomiyama, 1974). Despite species differences in TCE kinetics, comparable exhaled breath levels have been observed in rats and humans postexposure. Accounting for differences in exposure concentration, the postexposure exhaled breath levels of TCE from several studies in humans (Kimmerle and Eben, 1973a; Stewart *et al.*, 1974; Monster *et al.*, 1979) were similar in magnitude to the values of TCE eliminated in the exhaled breath of rats following inhalation exposure in the current investigation. Stewart *et al.* (1974), for example, measured concentrations of 0.70 and 0.28 ppm TCE in the exhaled breath of human subjects 30 and 120 min after termination of a 3-hr, 20 ppm inhalation exposure. Assuming a linear scaledown of the 50 ppm data from the current study, the TCE concentrations in the expired air of rats at these two time-points would be 0.92 and 0.28 ppm, respectively. Such a similarity in the magnitude of exhaled breath levels in humans and rats was also noted recently for 1,1,1-trichloroethane (Dallas *et al.*, 1989). The rat blood:air partition coefficient for each VOC is significantly higher than that for humans (Gargas *et al.*, 1989). Although this difference alone would result in greater respiratory elimination of the VOCs by humans, it is apparently offset by other factors, including the higher respiratory and circulatory rates of the rat.

PB-PK models for TCE have been developed by several groups of investigators. Sato *et al.* (1977) formulated a PB-PK model for respiratory exposure of humans to TCE. The model included three compartments, with intercompartment exchange of TCE governed solely by intertissue diffusion. Metabolic and respiratory excretion were assumed to occur in the richly perfused tissue compartment. Fernandez *et al.* (1977) constructed a more complete PB-PK model, which accurately predicted respiratory elimination of TCE and cumulative urinary excretion of TCE metabolites in humans. This model included the three compartments of Sato *et al.* (1977), as well as a liver compartment with blood-flow-limited metabolism and a lung compartment for respiratory absorption and elimination of TCE. Andersen *et al.* (1987) used a PB-PK model analogous to that of Ramsey and Andersen (1984) to predict the influence of competitive metabolic inhibition on uptake of inhaled TCE in rats. Fisher *et al.* (1989) subsequently modified the Ramsey and Andersen (1984) model to simulate the kinetics of TCE and trichloroacetic acid in the pregnant rat following inhalation and ingestion of TCE. Additional compartments (i.e., mammary tissue, placenta, and fetus) were incorporated into the model, and allowance was made for certain physiological changes which occur during pregnancy. The PB-PK model of Fisher *et al.* (1989) provided a good representation of TCE and trichloroacetic acid levels mea-

sured experimentally in maternal and fetal blood at a limited number of times postexposure. This model has been extended recently to predict the kinetics of TCE and trichloroacetic acid in lactating rats and nursing pups (Fisher *et al.*, 1990).

The PB-PK model used in the current investigation accurately predicted the time-courses of TCE concentrations in the blood and exhaled breath of rats during and following inhalation exposure to 50 and 500 ppm TCE. The model is similar to those of Ramsey and Andersen (1984) and Angelo and Pritchard (1984). Our PB-PK model differs in that it includes a separate lung tissue compartment and a lung:alveolar mass transfer coefficient, which describes the bidirectional transfer of TCE across the alveolar membrane. It is only necessary to alter the experimentally determined inhaled concentration and minute volume in order to obtain simulations of TCE kinetics under different inhalation exposure scenarios. Metabolic saturation, manifest by the progressive, disproportionate increase in blood levels in the high-dose (i.e., 500 ppm) animals, was accurately forecast. There was also good agreement between predicted and observed blood and breath levels during most of the postexposure period. Previous investigators, including Fisher *et al.* (1989), have had the use of very limited experimental data sets for assessing the precision of their model predictions.

Health risk assessments of VOCs such as TCE require a careful selection of the measure of dose. Areas under blood and tissue concentration versus time curves have been advocated as logical measures of target organ dose (Andersen, 1987). The most appropriate chemical species to measure for TCE depends upon which toxic effect is of interest. TCE appears to be primarily responsible for CNS depression and cardiac arrhythmias. As it is unclear which metabolite(s) should be used as dose measures, or surrogates for TCE-induced cytotoxicity and mutagenicity/carcinogenicity, the amount of reactive intermediate (i.e., toxicologically effective dose) has been equated to the total amount of TCE metabolized by the liver (Bruckner *et al.*, 1989). The NRC (1986) applied the PB-PK model of Ramsey and Andersen (1984) to calculate the toxicologically effective dose formed in the liver of rats during TCE inhalation exposure. Bogen (1988) has more recently applied the model of Ramsey and Andersen (1984) to predict relationships between the administered dose and the toxicologically active, or metabolized dose of TCE in humans. Comprehensive TCE time-course data sets, however, have not been available for rigorous validation of model predictions. The next logical step in this direction will be to obtain TCE/metabolite tissue concentration versus time data sets through direct measurement studies.

ACKNOWLEDGMENTS

The authors are grateful to Ms. Joy Wilson and Mrs. Judy Bates for their expertise in the preparation of this manuscript, and to Miss Elizabeth Lehman for her collation and recording of data.

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/ J14 C8794$$$35 07-05-91 14-57-59 AP: Toxicology — TOX6587

APPENDIX C

MANUSCRIPT TO BE SUBMITTED TO
TOXICOLOGY AND APPLIED PHARMACOLOGY

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "Comparative pharmacokinetics of inhaled and ingested 1,1-dichloroethylene in rats." To be submitted to Toxicology and Applied Pharmacology, 1991.

Comparative Pharmacokinetics of Inhaled and Ingested 1,1-Dichloroethylene in Rats

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Volatile organic chemicals (VOCs) are of concern to public health due to potential human exposures in both an occupational setting during their manufacture and from residential exposure due to subsequent contamination of drinking water supplies. The primary route to exposure in occupational environments is by inhalation, and until recently most prior interest in the health hazards of VOCs has been by inhalation exposure. Current toxicological knowledge of volatile organics is thus largely based upon situations and experiments involving inhalation exposures. In light of the increasing detection of these halocarbons in drinking water supplies in the United States (Symons et al., 1975, NOMS, 1977), there is ow considerable interest in the toxicity of these contaminants following oral exposure.

It is unclear, however, whether the results of inhalation studies can be used to accurately predict the consequences of ingestion of the chemicals. In one report (NAS, 1980), the use of inhalation data was avoided in making risk assessments of drinking water contaminants, with the reasoning that the disposition ad ensuing bioeffects of inhaled chemicals may differ markedly from that which occurs when the agents are ingested. It was concluded that while inhalation studies may be of value from a quantitatively in predicting consequences of ingestion of many chemicals. In contrast, the model by Stokinger and Woodward (1958) has been applied to use inhalation data to derive adjusted acceptable daily intake (ADI) for several short-chain aliphatic halocarbon VOCs (Federal Register, 1984). Unfortunately, there is a limited pharmacokinetic and toxicological data base from which to judge the validity of such route to route extrapolations.

One VOC of particular concern for potential oral and inhalation exposures in humans is the short-chain halocarbon 1,1-dichloroethylene (vinylidene chloride, DCE). DCE is of interest due to a variety of modes of toxicity such as hepatotoxicity (Jenkins, et al., 1972, Reynolds et al., 1975; Andersen et al., 1979), nephrotoxicity (Jenkins and Andersen, 1978; NIP, 1982), and carcinogenicity in one study (Maltoni et al., 1977). DCE has been measure at low levels (< 0.2 μg/liter) in well water and municipal drinking water supplies (U.S. EPA, 1975; Shakelford and Keith, 1976). However, groundwater contamination has been detected at considerably higher levels (Page, 1981; U.S. EPA, 1982). Comparable doses of DCE have been administered to rats by inhalation (McKenna et al., 1978a) and by gastric intubation (McKenna et al., 1978b). The question of influence of routes of administration on tissue disposition was not addressed directly. Emphasis was placed on the roles of dose and

fasting on metabolism, elimination and toxicity. However, tissue levels were measured at only a single time-point post exposure (i.e. 72 hours). 1,1-DCE has been shown to markedly alter calcium homeostasis in hepatocytes of rats within 20 to 30 minutes of dosing (Moor, 1982; Luthra et al., 1984). Overt cytotoxicity has been observed as early as 2 hours, with maximal injury manifest within 4 to 8 hours of 1,1-DCE exposure by inhalation (Reynolds et al., 1980) or by ingestion (Jaeger et al., 1973). Therefore, it is essential that the kinetics of DCE be compared between the two routes of administration during the critical early hours when cytotoxicity occurs.

The approach utilized in the current study was to administer equivalent doses of DCE orally and by inhalation in unanesthetized rats within the same time frame. Detailed physiologic measurements conducted during DCE inhalation exposures were used to determine the total uptake of DCE over time, accounting for the significant respiratory elimination of the inhaled compound. An equivalent dose was then administered to rats by single and multiple oral bolus dosing regimens and the systemic uptake, disposition, and elimination of DCE by the two administrative routes compared. A physiologically-based pharmacokinetic model for inhalation and oral DCE exposures was validated by comparison to the observed data and evaluated for use in interroute extrapolation of pharmacokinetic data.

MATERIALS AND METHODS

Animals. Adult, male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. They were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided ad libitum. The rats were used after at least a 14-day acclimation period, at which time their body weight ranged from 325-375 g. Solvent exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test material. 1,1-Dichloroethylene (DCE), of 99% purity, was obtained from Aldrich Chemical Co. (Milwaukee, WI) and the purity verified by gas chromatography prior to use in animal exposures.

Animal preparation. All rats were surgically prepared with an indwelling carotid arterial cannula, which exited the animal at the back of the neck. The rats were anesthetized for the surgical procedure by an injection

of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml): acepromazine maleate (10 mg/ml): xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v:v:v). The cannulated animals were maintained in metabolism cages with total freedom of movement during a 24-hr recovery period. For animals requiring intravenous administration, animals were also prepared with an indwelling jugular cannula simultaneously with the arterial cannula. For gastric infusion experiments, a ventral incision was made and a flared-tip cannula inserted into the fundus of the stomach simultaneously with carotid arterial surgery and exited through the same port as the arterial cannula.

Inhalation exposures. A known concentration of the test chemical was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of the solvent into the bag filled with air. Uniform dispersion of the vapor was insured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, a miniaturized one-way breathing valve (Hans Rudolph, Inc. St. Louis, MO), and an empty 70-l gas collection bag. The latter bag served as a reservoir to collect exhaled gas. The breathing valve was attached to a face mask designed to fit the rat so that the valve entry port was directly adjacent to the nares of the test animal, thus establishing separate and distinct airways for the inhaled and exhaled breath streams. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. A cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneve, Switzerland) and the face mask held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. DCE inhalation exposures were initiated only after stable breathing patterns were established for the cannulated animals in the system. The test animals then inhaled DCE vapors for a 2-hr period. During this exposure period and for up to 3 hr afterward, inhaled and exhaled breath samples were taken from the sampling ports at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for DCE content by gas chromatography.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored according to the methods previously published in solvent exposure studies by this laboratory (Dallas et al., 1983 and 1986). The airflow created by the animal's inspiration was recorded both during and following DCE

inhalation exposure in terms of minute volume (volume of respiration per minute, or V_E), respiratory rate (f), and tidal volume (V_T). An average value for these parameters for each individual animal was obtained by averaging the measurements taken at 10-min intervals during the 2-hr exposure. The mean ± SE of these average values for the 100 ppm exposure group (n=6) were: V_E 229.5 ± 8.9 ml/min; f = 136.2 ± 2.9 breaths/min; and V_T = 1.72 1 0.12 ml. The mean ± SE of these average values for the 300 ppm exposure group (n=6) were: V_E = 215.7 ± 18.8 ml/min; f = 134.5 ± 11.5 breaths/min; and V_T = 1.60 ± 0.09 ml.

Calculations of DCE uptake and elimination from inhalation exposure were conducted according to equations presented in previous pharmacokinetic determinations of TRI inhalation in rats by this laboratory (Dallas et al., 1988). Since the V_E and the TCE exhaled breath concentrations at each sampling point were measured, subtraction of the quantity of TCE exhaled from the animal from the amount inhaled yielded an approximation of the quantity of uptake of TCE for each sampling period (cumulative uptake). In the determination of the cumulative elimination of TCE during inhalation exposure, the cumulative uptake for each time interval was subtracted from the total inhaled dose for that interval. The percent uptake of the total inhaled dose up to each successive time point during the inhalation exposure period was determined by dividing the cumulative uptake by the inhaled dose for that time period.

Oral and intravenous dosage regimens. The value for the total cumulative uptake achieved for a two hour inhalation exposure was then used for the administration of an equivalent oral dose using three different regimens. A single oral bolus of 30 mg/kg (equivalent to 300 ppm DCE for 2 hr) and 10 mg/kg (equivalent to 100 ppm for 2 hr) was thus administered to unanesthetized rats with an indwelling carotid arterial cannula. The DCE was administered in an aqueous emulsion, Emulphor, intragastrically with a blunt-tipped intubation needle. For the multiple oral bolus administrative regimen, the 10 and 30 mg/kg doses were each subdivided into 4 equal doses and administered sequentially at 30-min intervals in Emulphor. For intragastric infusions, the animals previously prepared with an intragastric cannula received the 10 or 30 mg/kg dose in Emulphor over a 2-hr infusion period. The infusion was conducted using a microprocessor-controlled P22 syringe infusion pump (Harvard Apparatus, No. Natick, MA). The intravenous administration of DCE was conducted using the animals previously prepared with an indwelling jugular vein cannula. The intravenous doses were delivered as a single

bolus in PEG 400 through the jugular cannula. Blood samples were taken during and following the oral and iv administration of DCE from the indwelling carotid arterial cannula.

Analysis of DCE in air and blood. The concentration of TCE in the inhaled and exhaled air during the inhalation exposures were measured with a Tracor MT560 gas chromatograph (Tracor Instruments, Austin, TX) using an electron capture detector (ECD). Air samples were procured with a gas-tight, 1-ml syringe and injected directly onto an 8-ft X 1/8-in stainless-steel column packed with 0.1% AT 1000 on GraphPak. Standards were prepared in each of four 9-liter standard bottles which have Teflon® stoppers containing needles used for taking the air samples with the syringe. Operating temperatures were: 150°C, injection port; 360°C; ECD detector; 70°C, isothermal column operation. Gas flow rates were employed of 40 ml/min for nitrogen (carrier gas), with an additional make-up gas flow rate to the detector of 30 ml/min.

DCE levels in the blood from inhalation an iv administration and the various oral dosing regimens were measured by gas chromatographic headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock by a 1-ml syringe. Depending on the anticipated blood concentration, between 25 and 200 μl of the blood was taken from the stopcock with an Eppendorf pipette and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE lined butyle rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 autosampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer, Norwalk, CT), where it was heated to a preset temperature by a high precision thermostat device. A precise volume of the vapor was then injected automatically into the column for analysis. The column used was an 8-ft X 1/8 in stainless-steel column packed with FFAP Chromasorb W-AW (80-100 mesh). Operating temperatures were: 200°C, injection port; 360°C, ECD detector; and column oven, 70°C. The carrier gas was 5% argon-methane, at a flow rate of 40 ml/min with a make-up gas flow rate to the detector of 20 ml/min.

RESULTS

While 100 and 300 ppm were the target DCE inhalation concentrations, the actual concentration inhaled by the animals was determined by measurements of air samples taken from the airway immediately adjacent to

the breathing valve. Inhaled DCE concentrations for the six rats in each group were 310.0 ± 3.5 ppm for the 300 ppm exposures and 101.6 ± 0.8 ppm for the 100 ppm exposures.

During and following DCE inhalation, concentrations of the parent compound were measured in the exhaled breath (Fig. 1) and 100 ppm and 300 ppm exposed rats. Significant respiratory eliminations of unchanged DCE was evident during the inhalation exposure period, with steady-state DCE levels achieved in the exhaled breath within 20 min at both dose levels. DCE respiratory elimination was proportional to the inhaled concentration during exposure, as indicated by the exhaled breath values during 30-120 min of the exposure period (near steady-stat) of 71.6 ± 4.1 ppm and 204.6 ± 9.3 ppm (x ± SE) for the 100 and 300 ppm exposure groups, respectively. Upon cessation of DCE inhalation, the concentration of DCE declined very rapidly in the expired air of both exposure groups.

Measurements of the cumulative uptake of DCE by the rats (Fig. 2) was made by accounting for the quantity of unchanged DCE that was exhaled during the inhalation exposure period. As a result of the 2-hr exposure to 100 ppm DCE the cumulative uptake was 3.3 1 0.3 mg (x ± SE), or 10 mg/kg bw. The total cumulative uptake of DCE from the 2-hr exposure to 300 ppm was 10.2 ± 0 6 mg (x ± SE), or 30 mg/kg bw. Percent uptake of DCE during inhalation exposure was similar in magnitude at both exposure concentrations (Fig. 3). The % uptake decreased rapidly in the first 30 min to a near-steady state equilibrium thereafter. Mean values for % uptake during the second hr of exposure (near-stady state) were between 61 and 66% for both dose groups.

The cumulative elimination of DCE in the exhaled breath both during and following inhalation exposure is shown in Fig. 4. During DCE exposure, the cumulative elimination is dependent on DCE in the blood and on DCE eliminated from the alveolar and physiologic dead space that was not absorbed into the blood. The magnitude of pulmonary elimination was proportional to the inhalation exposure concentration. By the end of the 2-hr exposure to 100 and 300 ppm DCE, 2.2 ± 0.2 and 6.1 ± 1.3 mg (x ± SE), respectively, were eliminated from the rats in the exhaled breath. Following the termination of exposure, DCE that is eliminated in the breath is solely from unchanged DCE from the systemic circulation. During the 3-hr post-exposure period, an additional 0.14 and 0.37 mg of DCE were eliminated from the animals in the 100 and 300 ppm exposure groups, respectively.

Evaluation of the ratio of DCE concentration in the blood to DCE concentration in the exhaled breath over the duration of the inhalation exposure is shown in Fig. 5. After the first few minutes of exposure, this ratio was consistently higher for the 300-ppm-exposed rats relative to the 100 ppm group, though the difference is slight until the point at the termination of exposure (120 min.).

The arterial blood concentrations of DCE during and following inhalation exposure are compared to blood levels following equivalent oral doses using the three oral dosing regimens for a 300 ppm inhalation and 30 mg/kg oral dose (Figs. 6 a-c) and for a 100 ppm inhalation and 10 mg/kg oral dose (Fig. 7 a-c). After the initiation of inhalation exposure, substantial concentrations of DCE were found in the blood of all animals at the first sampling time (2 min). Uptake of DCE following single or multiple oral bolus administration was very rapid, as peak blood levels were achieved within 2 to 4 minutes of administration. Arterial DCE concentrations were not proportional to the inhalation concentration. After the initial rapid uptake phase over the first 20 minutes of exposure, blood levels for the 300 ppm-exposed rats were 4 to 5 times higher than DCE blood concentrations of rats that received 100 ppm exposures. The $x \pm SE$ for the blood concentrations from 30 to 120 min, during near-steady state, were 0.56 ± 0.03 and 2.19 ± 0.14 μg/ml for the 100 and 300 ppm exposure groups, respectively. At the high dose, peak blood levels were approximately 4 to 5 times higher following single oral bolus dosing than maximum blood levels (Cmax) achieved during the corresponding inhalation exposures (Fig. 6a). The maximum blood levels achieved during single oral bolus or gastric infusion administration were also not proportional to the administered dose. As with the inhalation exposures, these blood values for the high dose group were at least 4 to 5 times higher than for the low dose group for both oral administrative routes. The Cmax achieved for the multiple bolus administration of 30 mg/kg (Fig. 6c), however, was nearly 9 times more than that achieved following 10 mg/kg (Fig. 7c).

The AUC (area-under-the-blood-concentration-time curve) for each of the groups administered DCE orally was lower than the AUC seen for the rats inhaling an equivalent DCE dose by inhalation (Table 1). The AUC values for the single bolus and gastric infusion groups were similar at both dose levels (nearly identical for the high dose). These oral administration values were only 60-80% of the corresponding inhalation AUCs. The bioavailability (F) of DCE was determined by the ratio of the AUC value of each experimental group to the

corresponding dose administered by intravenous administration. The high dose groups consistently had a higher F than the groups administered the low dose of DCE by any of the exposure routes. At both the high and low doses, F was higher for animals inhaling DCE than for orally administered rats.

The terminal elimination half-life (t½) was similar for both inhalation exposure concentrations. Following a single oral bolus administration of DCE, the t½ was also similar to the corresponding inhalation exposure concentration. The administration of DCE by gastric infusion, however, resulted in two to three-fold increases in t½ relative to inhalation or single oral bolus DCE exposures. Apparent clearance and volume of distribution did not vary significantly with inhalation exposure concentration. Both of these pharmacokinetic parameters, however, were considerably lower for animals inhaling DCE than for the orally-administered rats.

DISCUSSION

Although there have been pharmacokinetic studies in which VOCs have been administered orally and by inhalation, the experimental design of most of the studies has been such that the results are of limited utility in making route extrapolations. Pyykko et al. (1977), for example, monitored the uptake and elimination of ^{3}H-toluene in various tissues of rats for 24 hours after the animals were dosed by inhalation or gastric intubation. As different doses of ^{3}H-toluene of different specific activity were given by each route of administration, no conclusions could be drawn about the relative uptake of the chemical by the two exposure routes. Typically, studies of the tissue disposition and binding of VOCs following inhalation and oral exposure involve oral administration of the compound as a single oral bolus, while inhalation exposures occur over a 6-hour period. As evidenced by this approach with perchloroethylene (Pegg et al., 1979) and 1,2-dichloroethane (Reitz et al., 1982), tissue concentrations and levels of covalently-bound ^{14}C are often measured only at a single time-point post-exposure (i.e. 72 hours). The relevance of these practices to interroute extrapolations of kinetic VOC data has thus been limited by questions of variations in the administered dose, the time elapsed between dosing and tissue analysis, and by the dissimilarity between the inhalation and oral dosing regimens.

Several approaches have been undertaken in the past in an attempt to utilize inhalation exposure data to estimate toxicity and acceptable levels of exposure to VOCs by ingestion. Some moderate correlations were

established in comparisons between inhalation LC_{50} data for single exposures to individual chemicals and mixtures (Pozzani et al., 1959). Stokinger and Woodward (1958) utilized the threshold limit value (TLV) for human inhalation of a chemical in workplace air in an equation for deriving a value that would represent an acceptable standard for ingestion exposure. Other variables incorporated were the retention, or absorption factor (% uptake), respiratory volume, and a safety (uncertainty) factor. An adaptation of this model for applications using animal inhalation data has been employed for estimating safe drinking water levels of perchloroethylene (Olson and Gehring, 1976). The accurate determination of the absorption factor (% uptake) has been recognized as essential to the extrapolation of dosages from inhalation data to the ingestion route (Khanna, 1983). It was pointed out that in previous interroute extrapolations, the absorption factor was either estimated based on physicochemical similarities with other agents, indirectly calculated from available data, or even unsupported by any rationale. In the present investigation, direct monitoring of minute volume and the magnitude of inhaled and exhaled DCE was utilized to determine the retention factor during inhalation exposures. This approach thus offers the advantage of direct measurements of respiratory volumes, the inhaled dose, and the retention factors that were previously estimated on a more tenuous basis in most interroute extrapolations.

The total cumulative uptake of DCE during inhalation exposures was determined from these summated measurements over time of the inhaled dose and the magnitude of pulmonary elimination. Administration of this amount as an equivalent oral dose to rats by several oral administrative routes reveled several differences in pharmacokinetics between these equivalent oral and inhalation exposures. Peak blood levels achieved after single oral bolus administration of DCE were 3 times higher than for the corresponding inhalation dose. Under the assumption that a minimum expression of certain kinds of acute toxicity for a chemical may require that a threshold level is achieved at a target organ, this finding would appear to attribute relatively greater risk to the oral bolus administration of DCE relative to the equivalent inhalation exposure. It is conceivable that a single high dose might produce injury by exceeding the metabolic detoxification pathway for the animal. Following its metabolic activation, DCE is inactivated by complexation with glutathione (Jones and Hathaway, 1978). However, there is evidence that a single high dose of certain halocarbons may be less toxic than the equivalent

dose given over a prolonged interval. Indeed, the findings of Chiesc et al. (1981) suggest that this may be the case for DCE. With a boiling point of only 31.7°C (Merck, 1976), DCE is one of the most volatile halocarbons. Together with its poor solubility in blood, this characteristic high volatility leads to a high rate of exhalation as a route of elimination for DCE. It is thus conceivable that a large portion of a bolus dose of DCE would be rapidly eliminated by exhalation before GSH stores are depleted to result in cytotoxicity. For inhalation exposures to DCE, however, it has been concluded that the metabolic capacity of rats to metabolize and eliminate DCE can be exceeded during continuous inhalation of 150 ppm or more of DCE (Dallas et al., 1983, Filser and Bolt, 1979). Therefore, despite higher peak blood levels from the single oral bolus administration relative to an equivalent inhalation dose, saturation of the metabolic capacity for DCE elimination and the subsequent increased risk for resulting toxicity may be more likely to occur from the inhalation exposure.

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TABLE 1

COMPARISON OF PHARMACOKINETIC PARAMETERS FOR INHALATION, ORAL, AND INTRAVENOUS ADMINISTRATIONS OF 1,1-DICHLOROETHYLENE IN RATS[a]

Dose/Administrative Route	AUC μg min/ml	F	t½ (min)	V_{dss} ℓ/kg	Cℓ (ml/ℓ min)
300 ppm Inhalation[b]	304.1±	0.58±	50.1±	0.091	4.0
30 mg/kg Gastric Infusion[b]	238.6	0.46	1/9.7	27.3	125.8
30 mg/kg Single Oral Bolus	239.5	0.46	62.1	6.3	125.3
30 mg/kg Multiple Oral Bolus[c]					
30 mg/kg Intravenous Bolus	519.4	---	164.5	10.8	136.8
100 ppm Inhalation	72.9	0.36	55.6	0.11	5.52
10 mg/kg Gastric Infusion	41.5	0.21	122.9	43.2	241.2
10 mg/kg Single Oral Bolus	50.0	0.25	47.2	10.4	20.0
10 mg/kg Multiple Oral Bolus[d]					
10 mg/kg Intravenous Bolus	202.6	---	105.7	6.82	49.4

a All values are x ± SD.

b Inhalation and gastric infusion administrations were over 2 hr.

c 30 mg/kg multiple oral bolus administered as three 10 mg/kg bolus doses at 40 min intervals.

d 10 mg/kg multiple oral bolus administered as three 3.3 mg/kg bolus doses at 40 min intervals.

Figure Legends

1. Exhaled breath concentrations of DCE during and following inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each point represents the mean ± SE for 6 rats.
2. Cumulative uptake of DCE during inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. The quantity of inhaled DCE retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled DCE concentrations. Each point represents the mean ± SE for 6 rats.
3. Percent uptake of DCE during inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each point represents the mean ± SE for 6 rats. The percent uptake of the inhaled dose over time was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter.
4. Cumulative elimination of DCE during following inhalation exposures. Rats inhaled 100 or 200 ppm DCE for 2 hr. The quantity of inhaled DCE eliminated in the breath over time was calculated using direct measurements of the minute volume and DCE concentrations in the inhaled and exhaled breath. Each point is the mean ± SE for 6 rats. Cumulative elimination was determined for successive 10-min intervals during the 2-hr exposure, and for successive 15-min intervals post-exposure.
5. Ratio of the DCE arterial blood concentration to the DCE exhaled breath concentration at each sampling point during inhalation exposure to DCE. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each observed value represents the mean ratio for 6 rats.
6. Comparison of DCE blood levels during and following 2 hr inhalation exposures to 300 ppm DCE with: a) blood levels following a single oral bolus of 30 mg/kg; b) blood levels following four repeated oral bolus administrations of 7.5 mg/kg each at 30 min intervals; c) blood levels during and following intragastric infusion of 30 mg/kg over a 2 hr period. Each value represents the mean ± SE for 6 rats.
7. Comparison of DCE blood levels during and following 2 hr inhalation exposures to 100 ppm DCE with: a) blood levels following a single oral bolus of 10 mg/kg; b) blood levels following four repeated oral bolus administrations of 2.5 mg/kg each at 30 min intervals; c) blood levels during and following intragastric

infusion of 10 mg/kg over a 2 hr period. Each value represents the mean ± SE for 6 rats.

Footnotes

1 Research sponsored by U.S. EPA Cooperative Agreement CR 812267 and the Air Force Office of Scientific Research, Air Force Systems Command, USAF, under grant number AFOSR 87-01248. The US Government is authorized to reproduce and distribute reprints for Governmental purposes notwithstanding any copyright notation thereon. This manuscript is submitted for publication with the understanding that the US Government is authorized to reproduce and distribute reprints for Governmental purposes.

2 Presented at the 27th Annual Meeting of the Society of Toxicology, Dallas, TX, February, 1988.

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Acknowledgment

The authors are grateful to Ms. Linda Quigley for her expertise in preparation of this manuscript and to Miss Elizabeth Lehman for her excellent technical assistance.

Index Terms

1,1-dichloroethylene

Vinylidene Chloride

Physiologically-based Pharmacokinetic Model

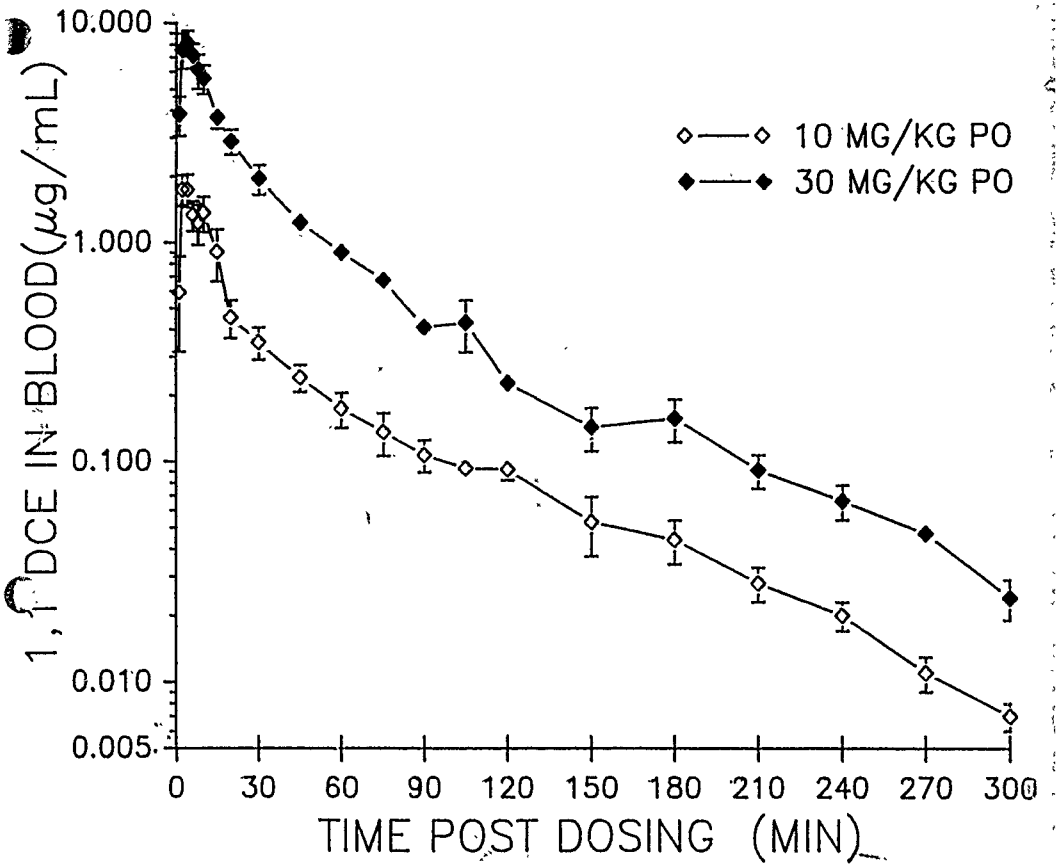
Respiratory Elimination

Pharmacokinetics

Inhalation Exposure

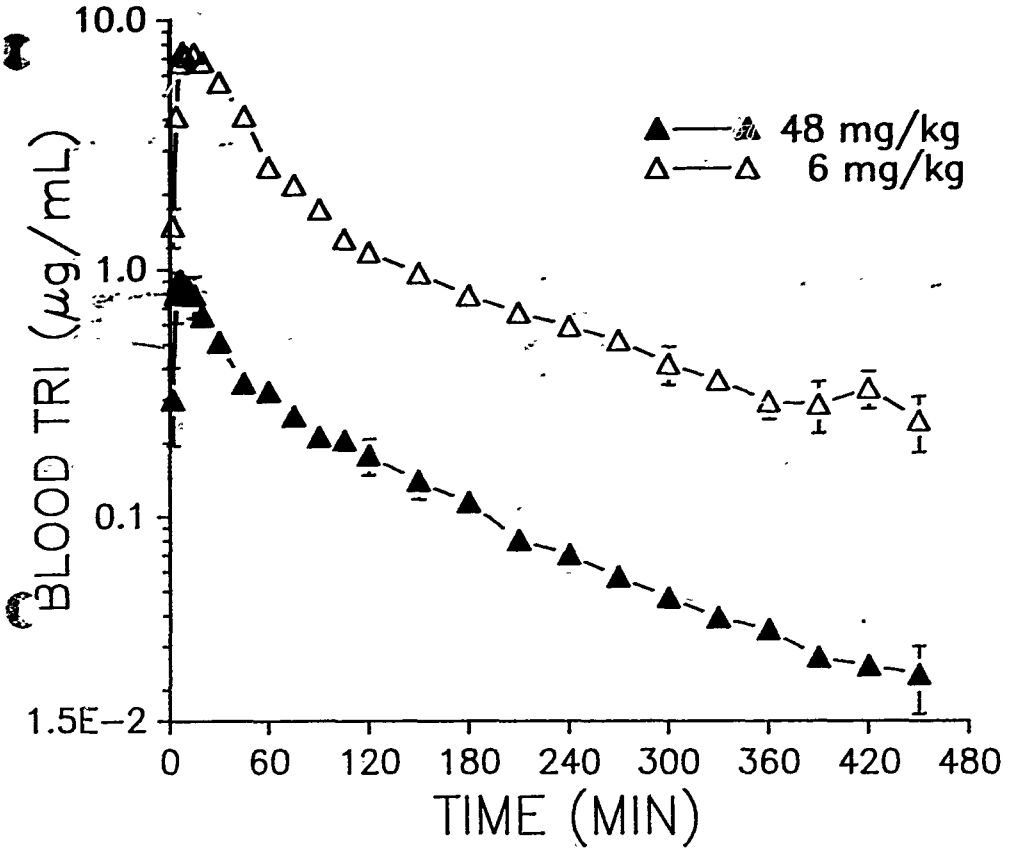
Oral Exposure

Interroute Extrapolation



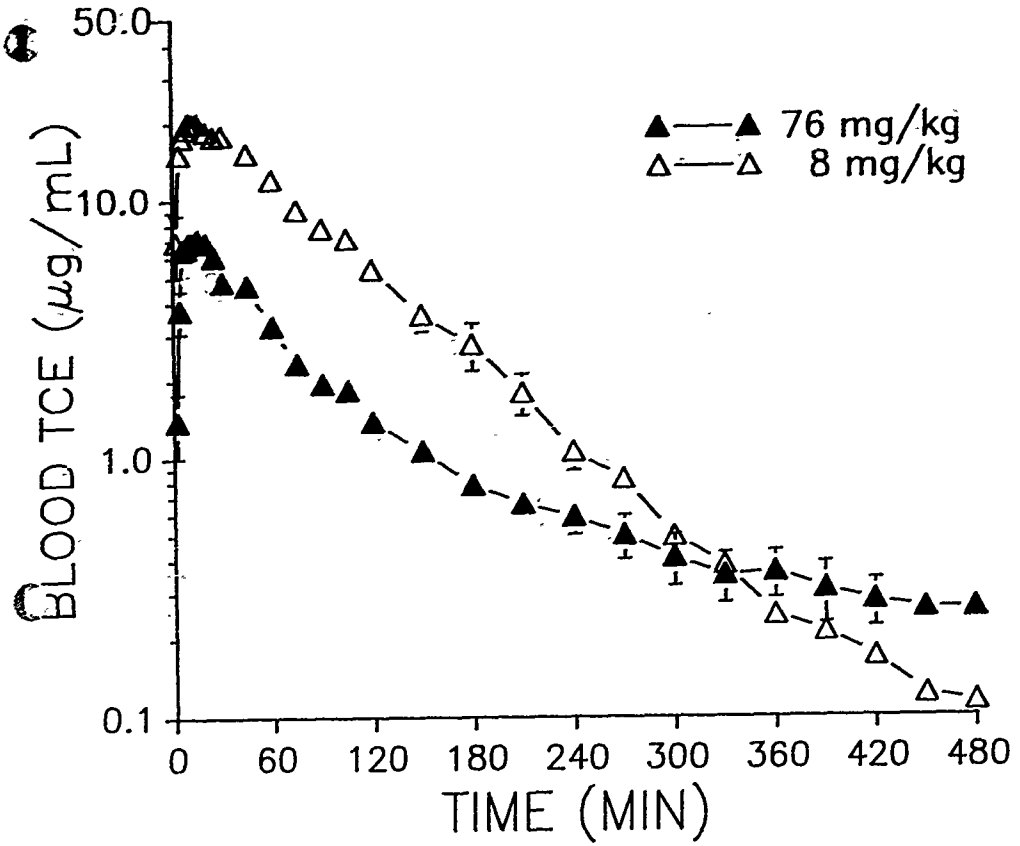
1,1-Dichloroethylene (DCE) concentrations in the blood of unanesthetized rats that have received a single oral bolus administration of 10 mg/kg or 30 mg/kg DCE in emulphor. Blood samples were taken from an indwelling carotid arterial cannula at 2 to 5 minute intervals during the rapid uptake phase and at 15 to 60 minute intervals during the elimination phase. Each value represents the mean ± SE for 6-8 rats.

Figure 8



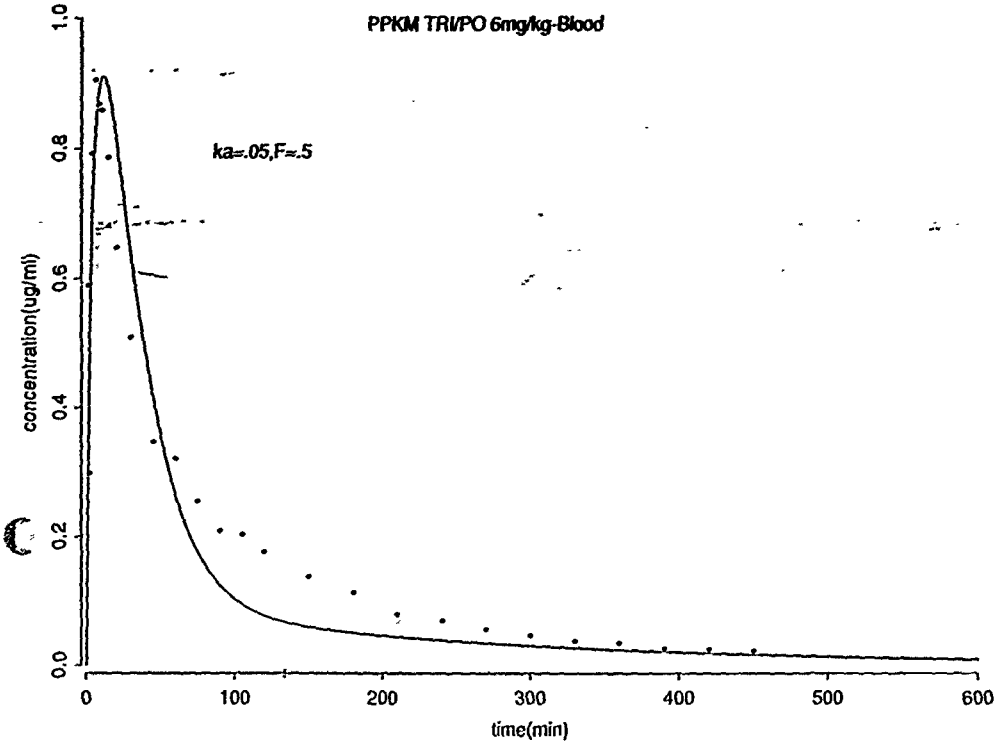
1,1-Trichloroethane (TRI) concentrations in the blood of unanesthetized rats that have received a single oral bolus administration of 6 mg/kg or 48 mg/kg TRI in emulphor. Blood samples were taken from an indwelling carotid arterial cannula at 2 to 5 minute intervals during the rapid uptake phase and at 15 to 60 minute intervals thereafter. Each value represents the mean ± SE for 6-8 rats.

Figure 9



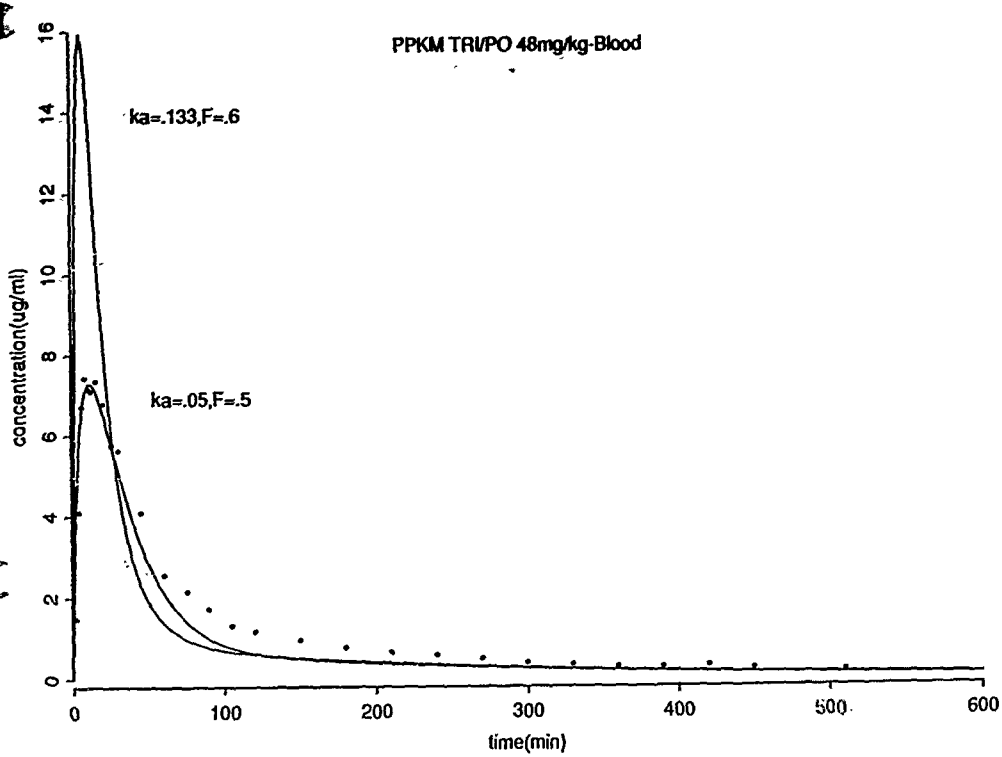
1,1,2-Trichloroethylene (TCE) concentrations in the blood of unanesthetized rats that have received a single oral bolus administration of 8 mg/kg or 76 mg/kg TCE in emulphor. Blood samples were taken from an indwelling carotid arterial cannula at 2 to 5 minute intervals during the rapid uptake phase and at 15 to 60 minute intervals thereafter. Each value represents the mean ± SE for 6-8 rats.

Figure 10



Observed (•) and model-predicted (-) TRI concentrations in the blood of rats that have received a single oral bolus administration of 6 mg/kg TRI in emulphor. In the physiologically-based pharmacokinetic model used for the simulation of TRI ingestion, a bioavailability of 0.5 and a Ka of 0.05 were employed. Each observed value is the mean for 8 rats.

Figure 11



Observed (•) and model-predicted (-) TRI concentrations in the blood of rats that have received a single oral bolus administration of 48 mg/kg TRI in emulphor. In the physiologically-based pharmacokinetic model used for the simulation of TRI ingestion, a bioavailability of 0.5 and a Ka of 0.05 were employed. Each observed value is the mean for 6 rats.

Figure 12

TABLE 1

COMPARISON OF PHARMACOKINETIC PARAMETERS FOR INHALATION, ORAL, AND INTRAVENOUS ADMINISTRATIONS OF 1,1-DICHLOROETHYLENE IN RATS[a]

Dose/Administrative Route	AUC μg min/ml	F	$t_{\frac{1}{2}}$ (min)	V_{dss} ℓ/kg	Cl_t (ml/ℓ min)
300 ppm Inhalation[b]	304.1±	0.58±	50.1±	0.091	4.0
30 mg/kg Gastric Infusion[b]	238.6	0.46	1/9.7	27.3	125.8
30 mg/kg Single Oral Bolus	239.5	0.46	62.1	6.3	125.3
30 mg/kg Multiple Oral Bolus[c]					
30 mg/kg Intravenous Bolus	519.4	---	164.5	10.8	136.8
100 ppm Inhalation	72.9	0.36	55.6	0.11	5.52
10 mg/kg Gastric Infusion	41.5	0.21	122.9	43.2	241.2
10 mg/kg Single Oral Bolus	50.0	0.25	47.2	10.4	20.0
10 mg/kg Multiple Oral Bolus[d]					
10 mg/kg Intravenous Bolus	202.6	---	105.7	6.82	49.4

a All values are x ± SD.

b Inhalation and gastric infusion administrations were over 2 hr.

c 30 mg/kg multiple oral bolus administered as three 10 mg/kg bolus doses at 40 min intervals.

d 10 mg/kg multiple oral bolus administered as three 3.3 mg/kg bolus doses at 40 min intervals.

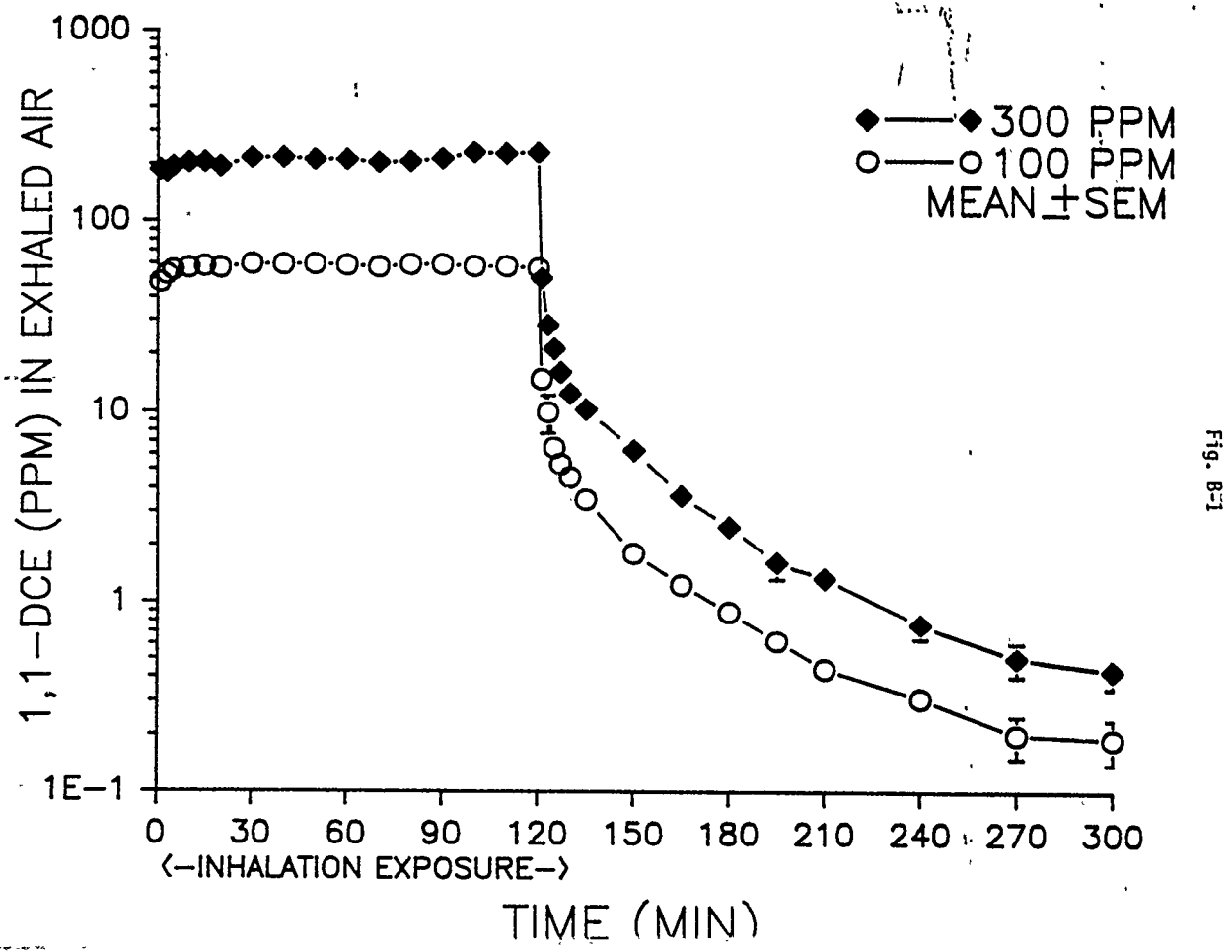

1000
100
10
1
1E−1
1,1−DCE (PPM) IN EXHALED AIR
300 PPM
100 PPM
MEAN ± SEM
0
30
60
90
120
150
180
210
240
270
300
<−INHALATION EXPOSURE−>
TIME (MIN)

Fig. 8-1

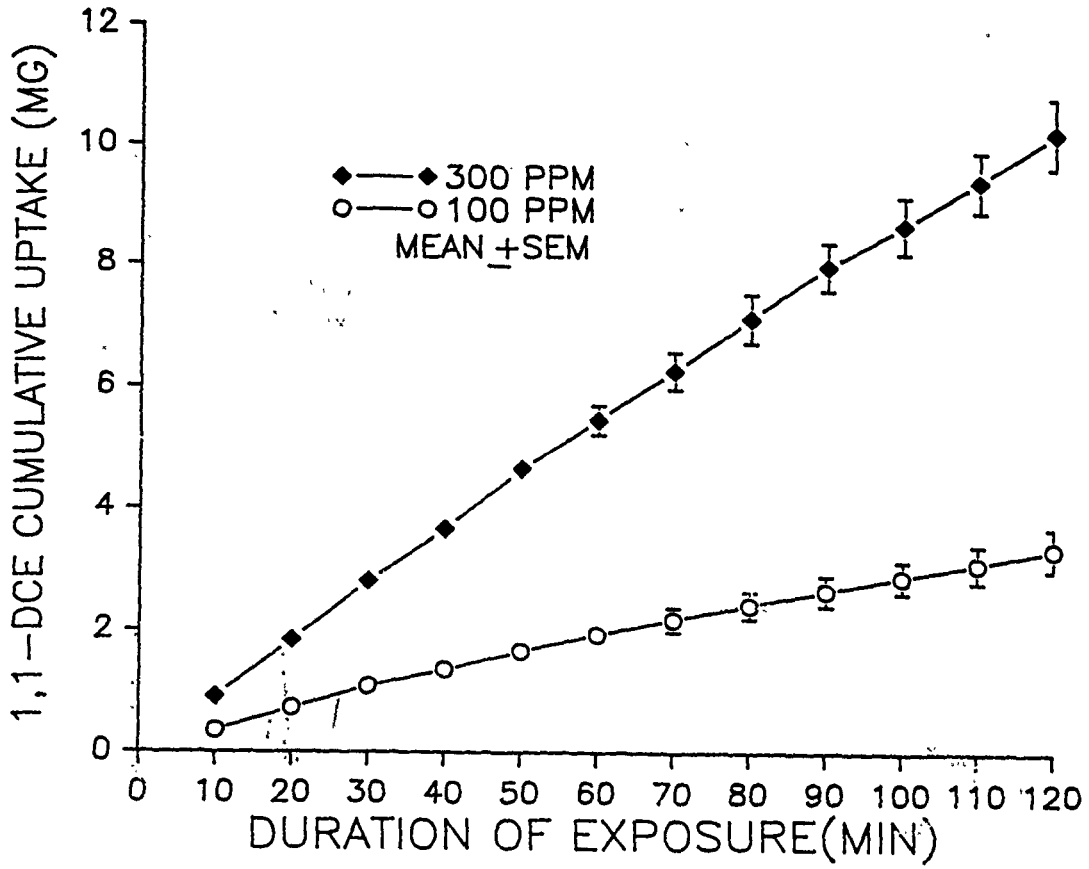

1,1-DCE CUMULATIVE UPTAKE (MG)
12
10
8
6
4
2
0
300 PPM
100 PPM
MEAN ± SEM
0 10 20 30 40 50 60 70 80 90 100 110 120
DURATION OF EXPOSURE(MIN)

Fig. B-2

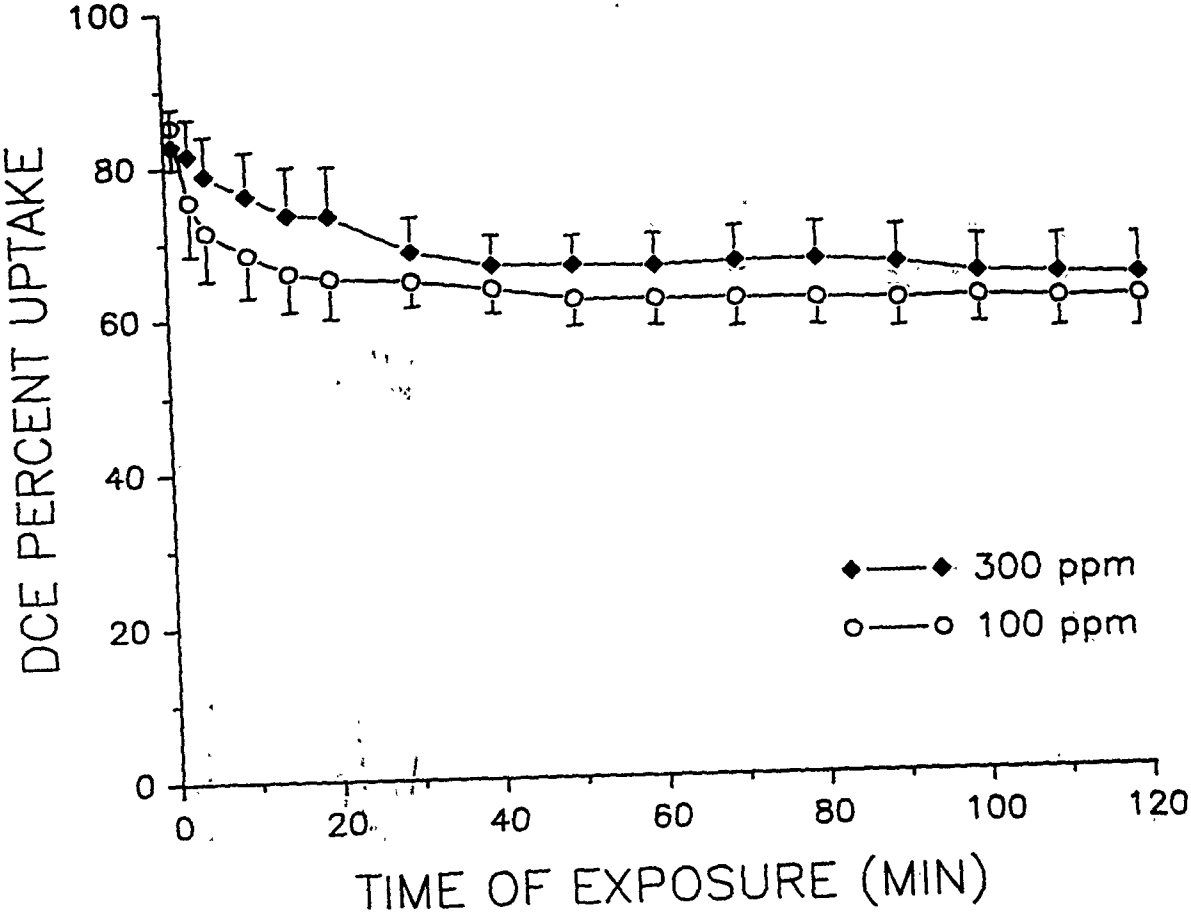

DCE PERCENT UPTAKE
100
80
60
40
20
0
0
20
40
60
80
100
120
TIME OF EXPOSURE (MIN)
300 ppm
100 ppm

Fig. B-3

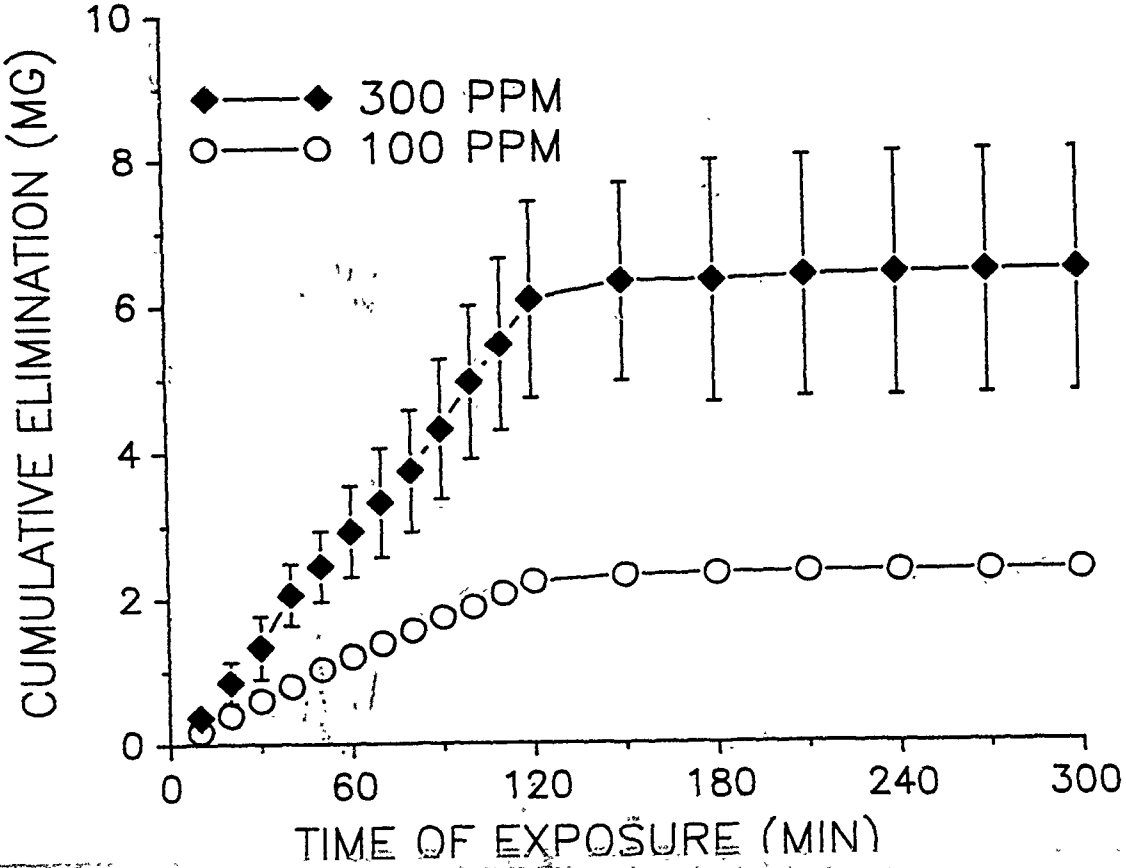

CUMULATIVE ELIMINATION (MG)
10
8
6
4
2
0
300 PPM
100 PPM
0
60
120
180
240
300
TIME OF EXPOSURE (MIN)

Fig. 8-4

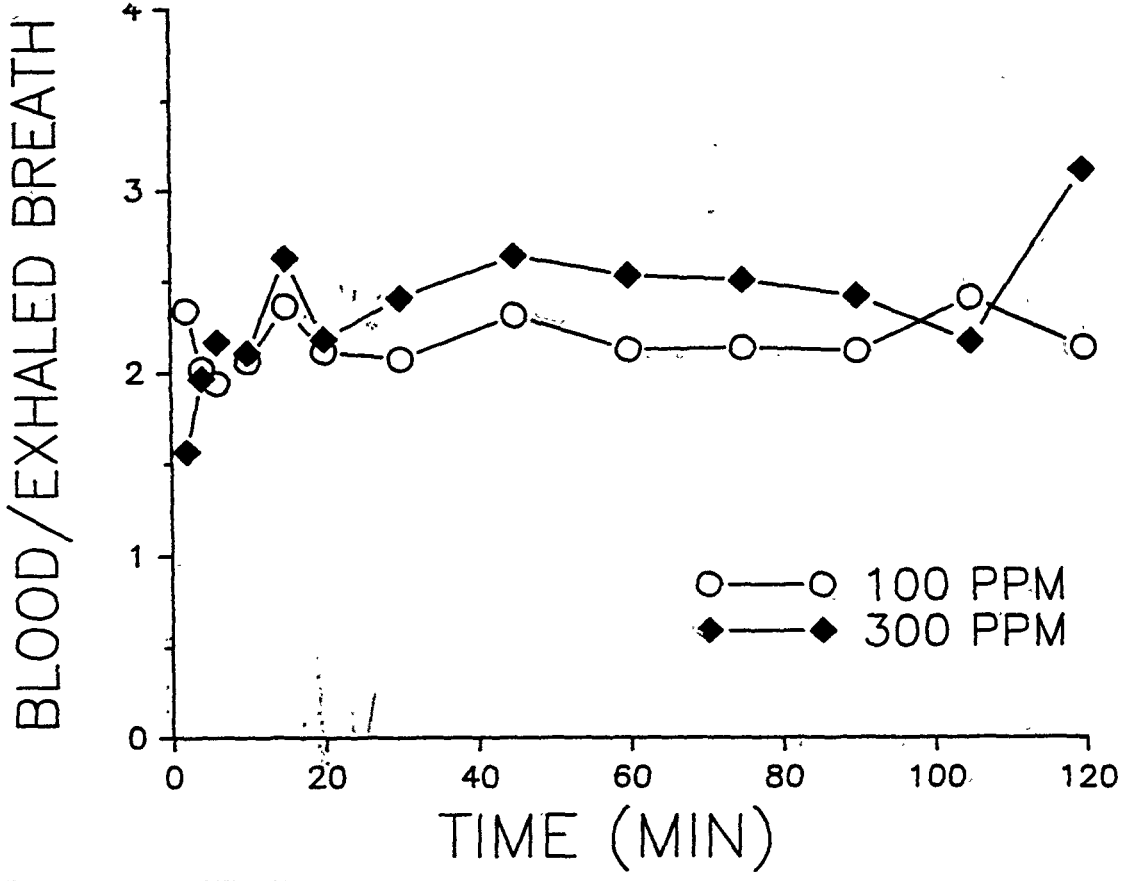

BLOOD/EXHALED BREATH
4
3
2
1
0
0
20
40
60
80
100
120
TIME (MIN)
100 PPM
300 PPM

Fig. B-5

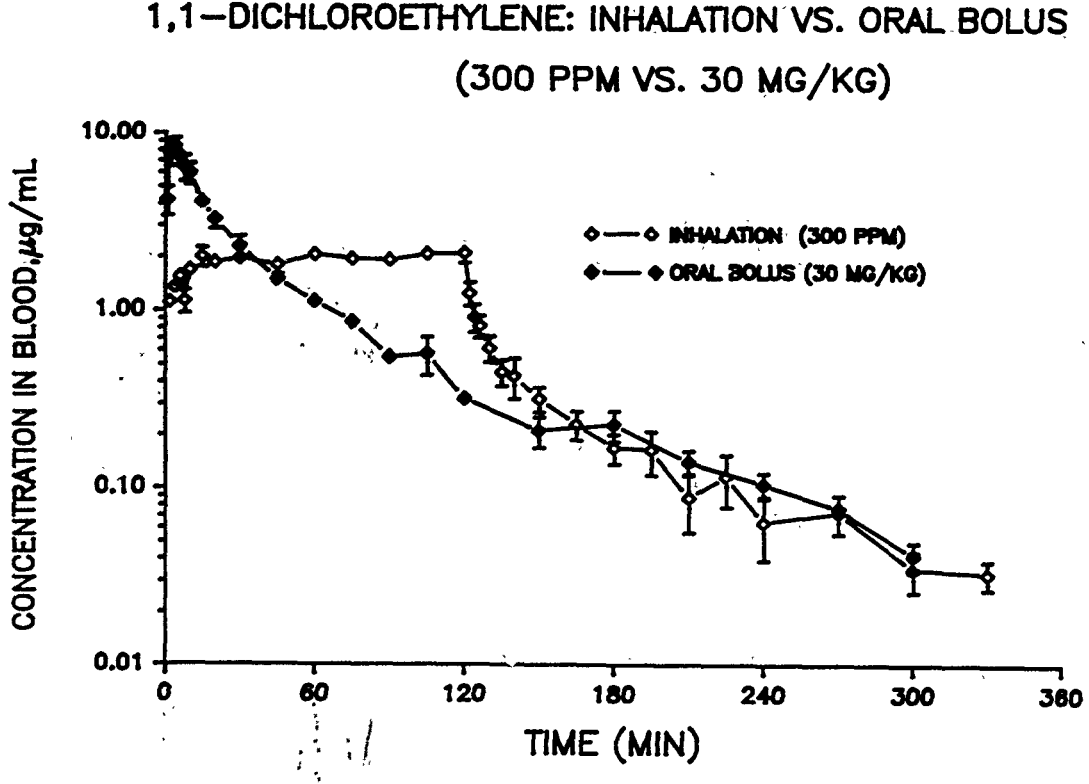

1,1—DICHLOROETHYLENE: INHALATION VS. ORAL BOLUS
(300 PPM VS. 30 MG/KG)
CONCENTRATION IN BLOOD, μg/mL
10.00
1.00
0.10
0.01
INHALATION (300 PPM)
ORAL BOLUS (30 MG/KG)
0
60
120
180
240
300
360
TIME (MIN)

Fig. B-6a

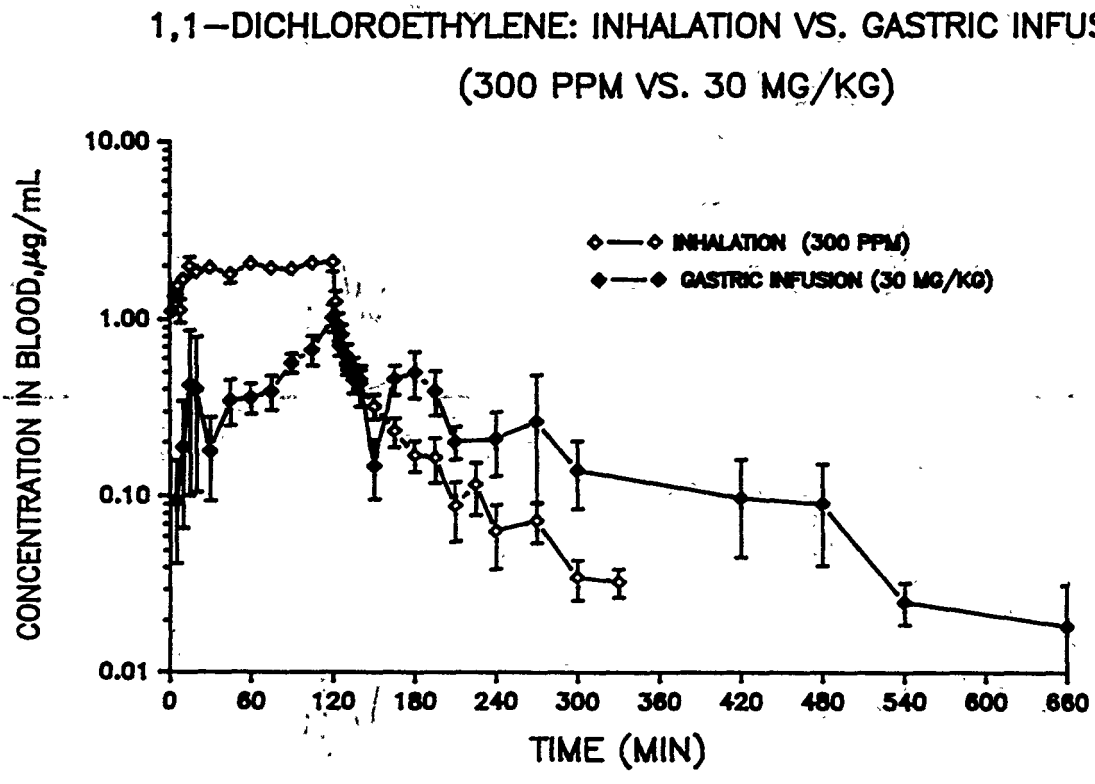

1,1—DICHLOROETHYLENE: INHALATION VS. GASTRIC INFUSION
(300 PPM VS. 30 MG/KG)
CONCENTRATION IN BLOOD,μg/mL
10.00
1.00
0.10
0.01
INHALATION (300 PPM)
GASTRIC INFUSION (30 MG/KG)
0
60
120
180
240
300
360
420
480
540
600
660
TIME (MIN)

Fig. B-6b

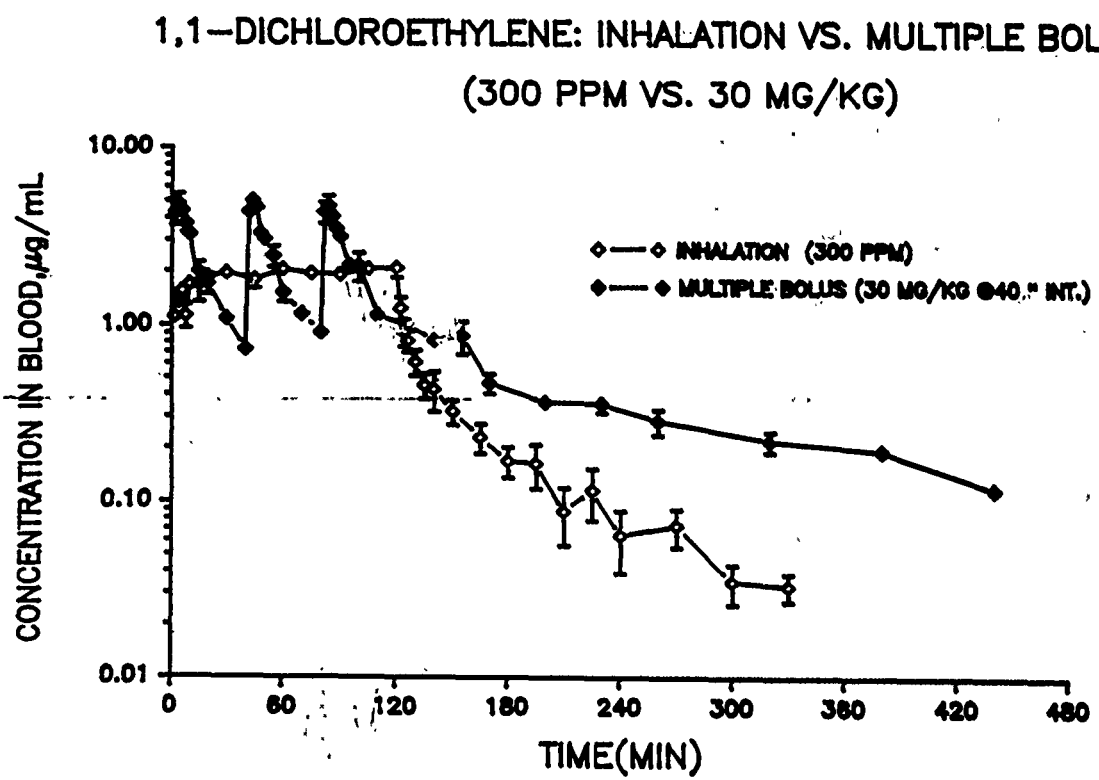

1,1—DICHLOROETHYLENE: INHALATION VS. MULTIPLE BOLUS
(300 PPM VS. 30 MG/KG)
CONCENTRATION IN BLOOD,μg/mL
10.00
1.00
0.10
0.01
0
60
120
180
240
300
360
420
480
TIME(MIN)
INHALATION (300 PPM)
MULTIPLE BOLUS (30 MG/KG @40.' INT.)

Fig. B-6c

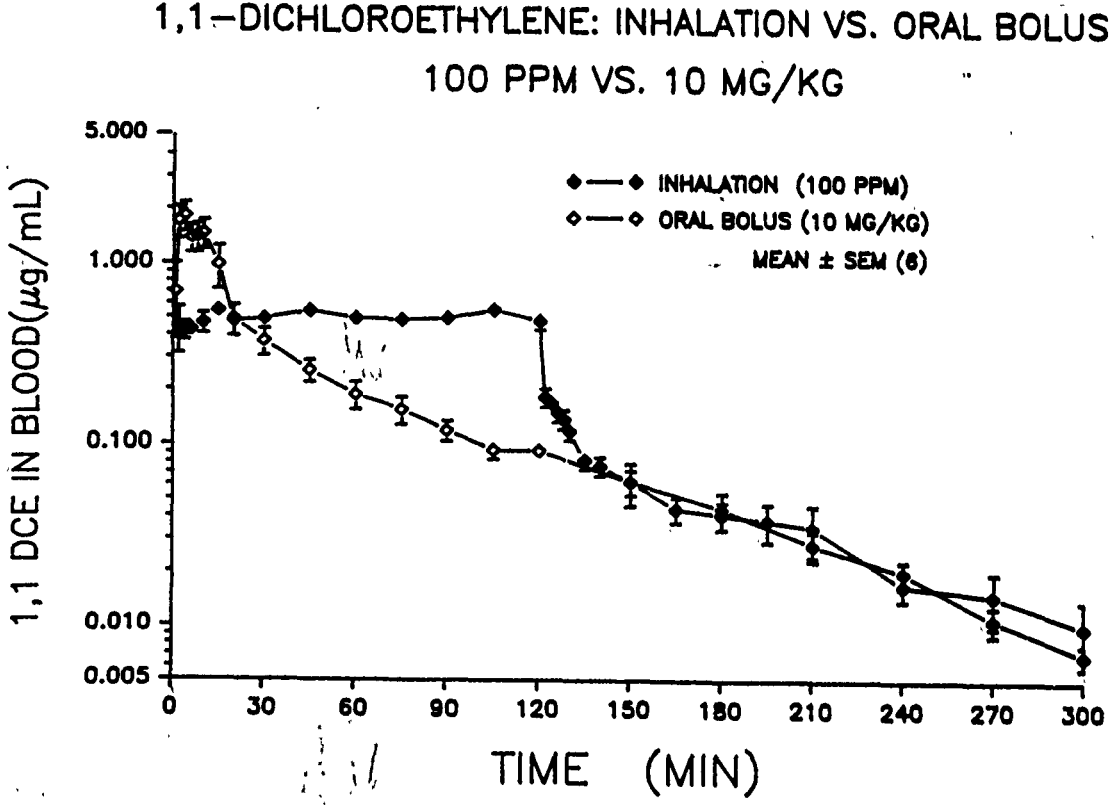

1,1–DICHLOROETHYLENE: INHALATION VS. ORAL BOLUS
100 PPM VS. 10 MG/KG
INHALATION (100 PPM)
ORAL BOLUS (10 MG/KG)
MEAN ± SEM (6)
1,1 DCE IN BLOOD(μg/mL)
5.000
1.000
0.100
0.010
0.005
0
30
60
90
120
150
180
210
240
270
300
TIME (MIN)

Fig. B-7a

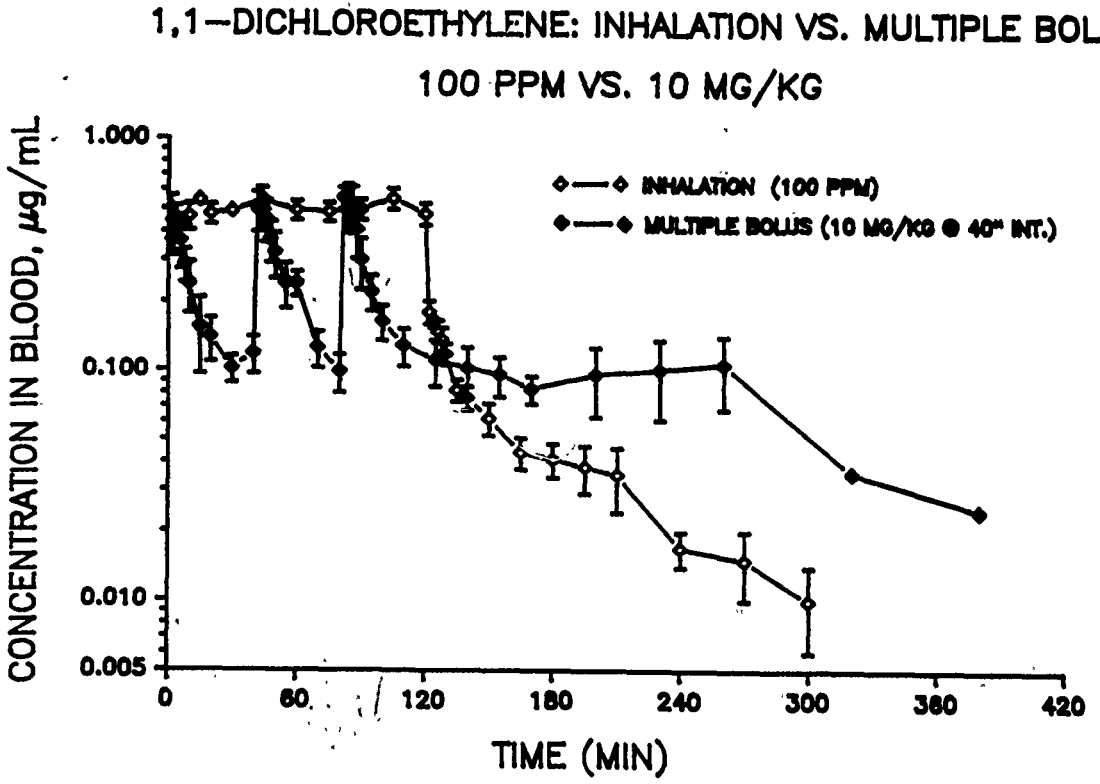

1,1—DICHLOROETHYLENE: INHALATION VS. MULTIPLE BOLUS
100 PPM VS. 10 MG/KG
CONCENTRATION IN BLOOD, μg/mL
1.000
0.100
0.010
0.005
INHALATION (100 PPM)
MULTIPLE BOLUS (10 MG/KG @ 40' INT.)
0
60
120
180
240
300
360
420
TIME (MIN)

Fig. B-7b

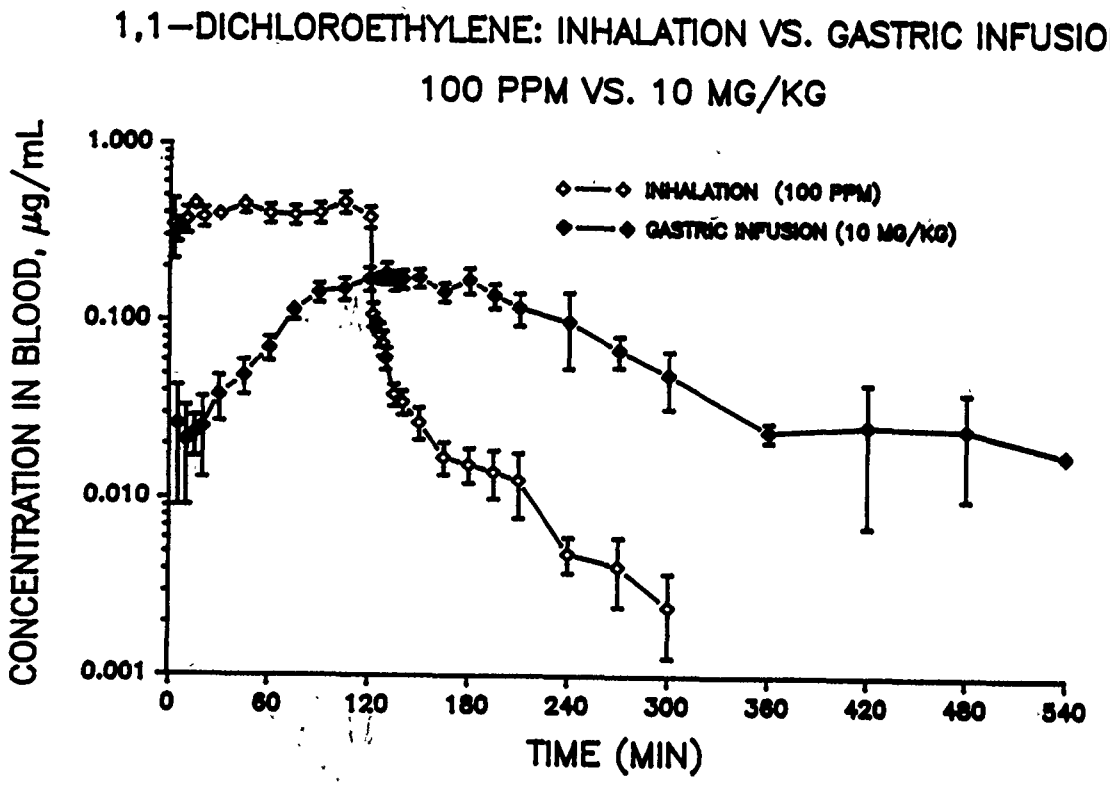
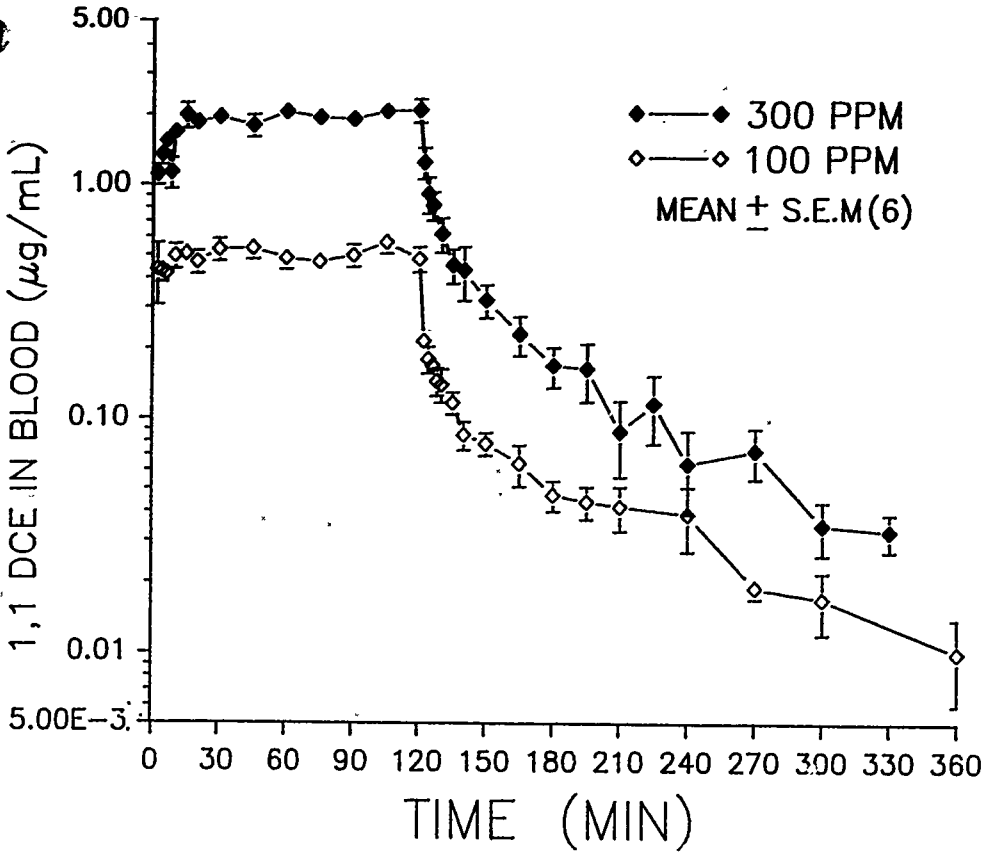
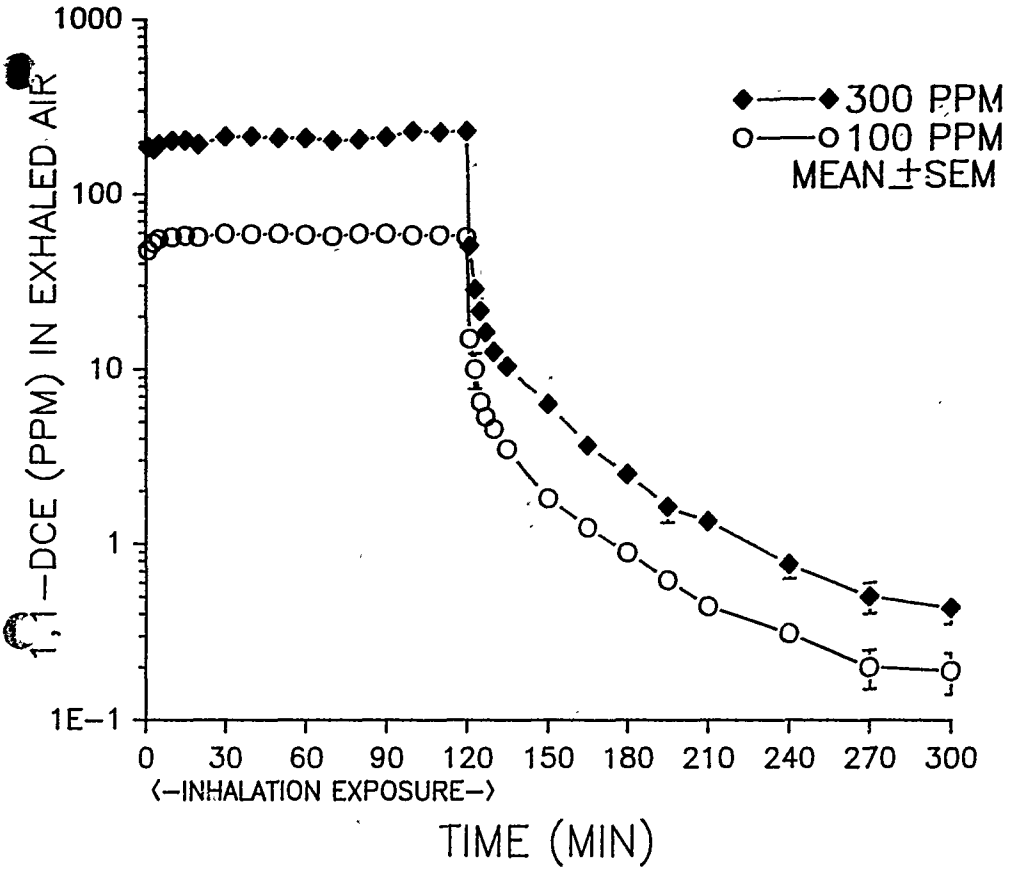

1,1—DICHLOROETHYLENE: INHALATION VS. GASTRIC INFUSION
100 PPM VS. 10 MG/KG
CONCENTRATION IN BLOOD, μg/mL
1.000
0.100
0.010
0.001
INHALATION (100 PPM)
GASTRIC INFUSION (10 MG/KG)
0
60
120
180
240
300
360
420
480
540
TIME (MIN)

Fig. B-7c



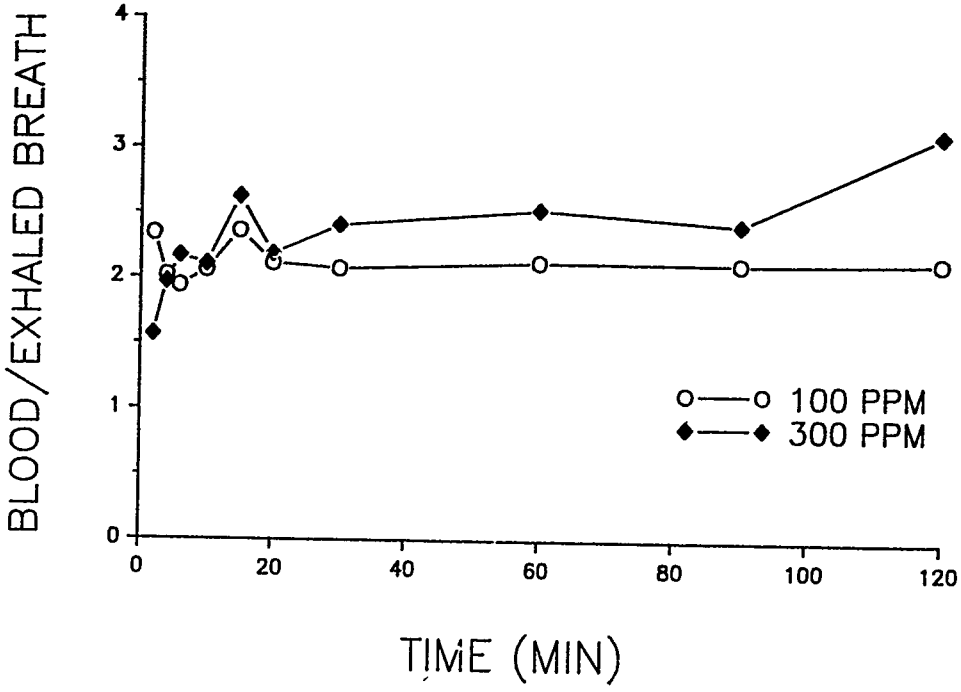
DCE concentrations in the blood of rats that have inhaled 100 or 300 ppm DCE for 2 hours. Levels of DCE were measured at 2 to 5 minute intervals during the rapid uptake phase (during exposure) and the rapid elimination phase (immediately following exposure) and at 15 to 60 minute intervals thereafter. Each value is the mean ± SE for 6 rats.

Figure 1



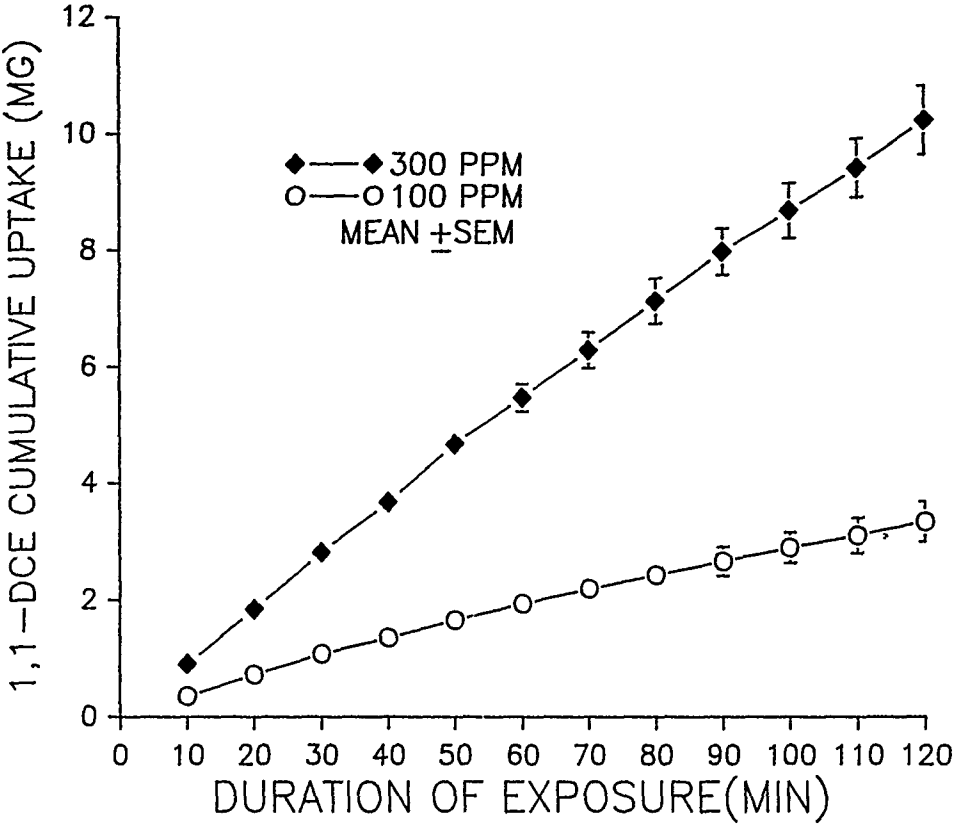
DCE concentrations in the exhaled breath of rats that have inhaled 100 or 300 ppm DCE for 2 hours. Levels of DCE were measured at 2 to 5 minute intervals during the rapid uptake phase (during exposure), and the rapid elimination phase (immediately following exposure) and at 15 to 60 minute intervals thereafter. Each value is the mean ± SE for 6 rats.

Figure 2



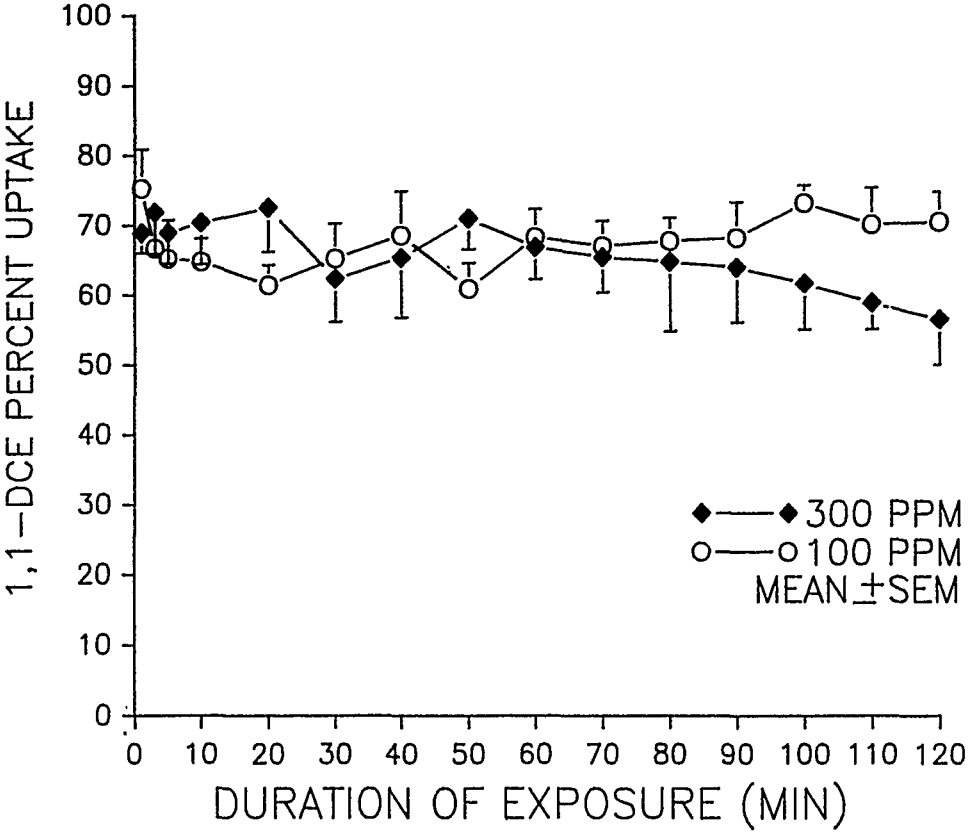
Ratio of the DCE arterial blood concentration to the DCE exhaled breath concentration at each sampling time point during inhalation exposure to DCE. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each observed value represents the mean ratio for 6 rats.

Figure 3



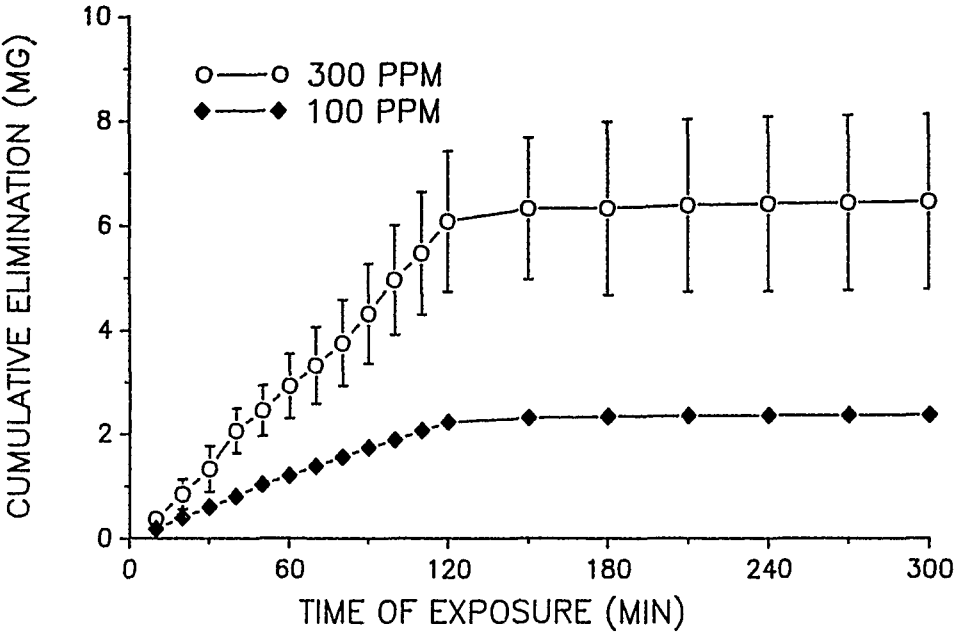
Cumulative uptake of DCE during inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. The quantity of inhaled DEC retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled TCE concentrations. Each point represents the mean ± SE for 6 rats.

Figure 4



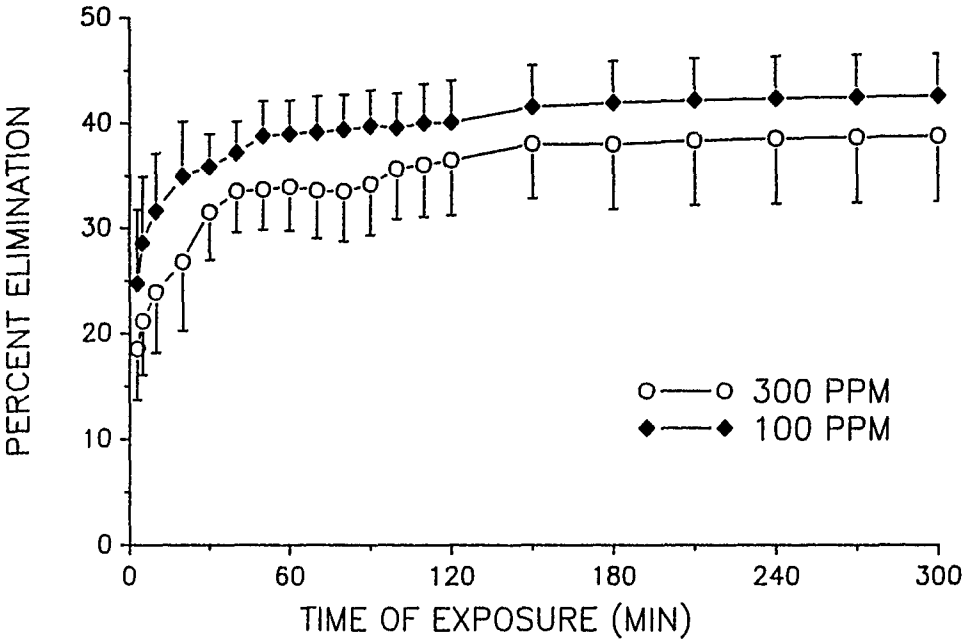
Percent uptake of DCE during inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each point represents the mean ± SE for 6 rats. The percent uptake of the inhaled dose over time was determined after 1, 3, 5, 10, and 20 min and at 10-min intervals thereafter.

Figure 5



Cumulative elimination of DCE during and following inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. The quantity of inhaled DCE eliminated in the breath over time was calculated using direct measurements of the minute volume and DCE concentrations in the inhaled and exhaled breath. The contribution of inhaled DCE from instrumental and anatomic dead space to the quantity exhaled was deleted. Each point is the mean ± SE for 6 rats. Cumulative elimination was determined for successive 10-min intervals during the 2-hr exposure, and for successive 15-min intervals post-exposure.

Figure 6



Percent elimination of DCE during and following inhalation exposures. Rats inhaled 100 or 300 ppm DCE for 2 hr. Each point represents the mean ± SE for 6 rats. The percent of the inhaled dose that was eliminated over time was determined after 3, 5, and 10 min and at 10-min intervals thereafter during exposure, and at 30-min intervals post-exposure.

Figure 7

APPENDIX D

PHARMACOKINETIC DATA FOR INGESTED TRI, TCE, AND DCE

TABLE I

PHARMACOKINETIC PARAMETERS FOR 1,1-DICHLOROETHYLENE (DCE) FOLLOWING SINGLE ORAL BOLUS ADMINISTRATION IN UNANESTHETIZED RATS

	DOSE	
	30 mg/kg	10 mg/kg
@C-MAX (UG/ML)	9.6 ± 1.3	2.25 ± 0.28
AUC (UG.MIN/ML)	239 ± 23.7	50.5. ± 5.9
ELI.HALF-LIFE (MIN)	55 ± 4.6	50 ± 3.6
APP.CLEARANCE (ML/MIN/KG)	131 ± 12	216 ± 23
APP.VOL.DIST (L/KG)	10.67 ± 1.9	16.1 ± 2.5

Values are the MEAN ± SE for 6 to 8 rats.

@ DCE in arterial blood.

TABLE II

PHARMACOKINETIC PARAMETERS FOR 1,1,1-TRICHLOROETHANE (TRI) FOLLOWING SINGLE ORAL BOLUS ADMINISTRATION IN UNANESTHETIZED RATS

	DOSE	
	48 mg/kg	6 mg/kg
@C-MAX (UG/ML)	7 ± 0.5	0.8 ± 0.07
AUC (UG.MIN/ML)	646 ± 35	64 ± 6
ELI.HALF-LIFE (MIN)	115 ± 7	112 ± 4
APP.CLEARANCE (ML/MIN/KG)	76 ± 3.7	91.7 ± 8.0
APP.VOL.DIST (L/KG)	12.1 ± 1.7	19 ± 0.9

Values are the MEAN ± SE for 6 to 8 rats.

@ Concentration of TRI in arterial blood.

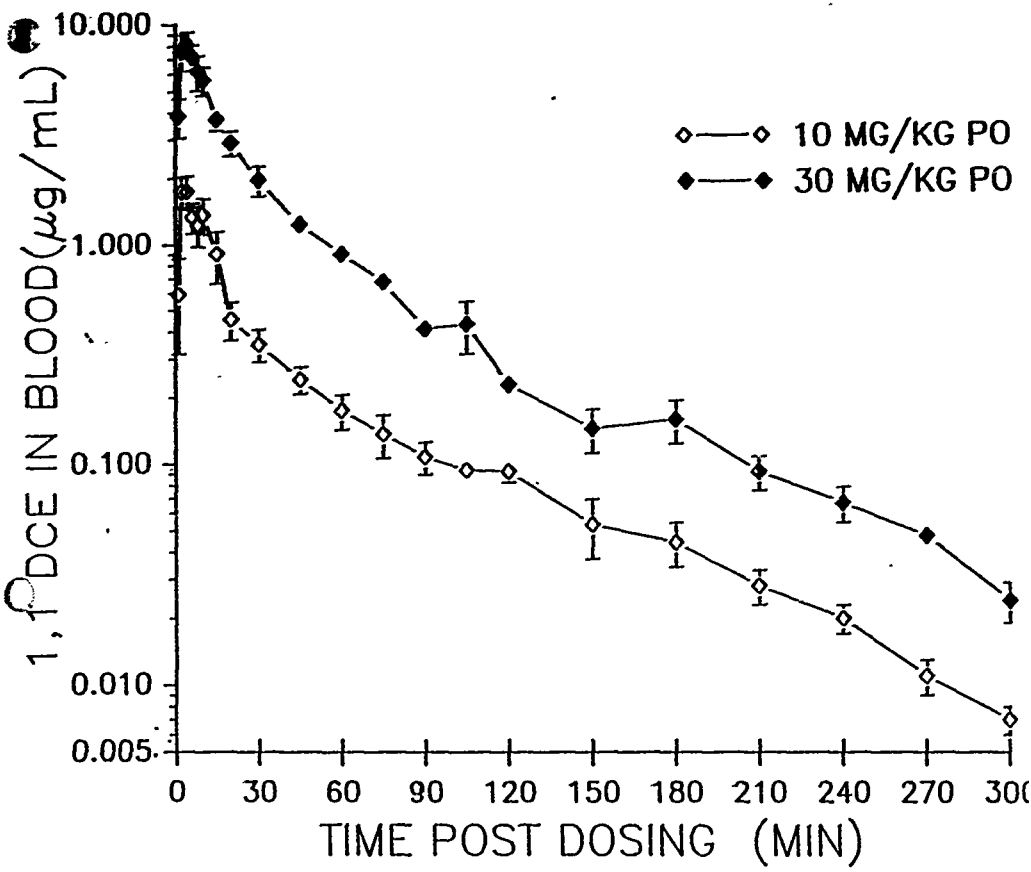
TABLE III

PHARMACOKINETIC PARAMETERS FOR TRICHLOROETHYLENE (TCE)
FOLLOWING SINGLE ORAL BOLUS ADMINISTRATION IN UNANESTHETIZED RATS

	DOSE	
	76 mg/kg	8 mg/kg
@C-MAX (UG/ML)	7.7 ± 0.8	1.50 ± 0.2
AUC (UG.MIN/ML)	936 ± 72	42.5 ± 2.8
ELI.HALF-LIFE (MIN)	116 ± 13	78 ± 1.6
APP.CLEARANCE (ML/MIN/KG)	85.6 ± 8.6	181 ± 16
APP.VOL.DIST (L/KG)	11.1 ± 1.0	21.7 ± 4.3

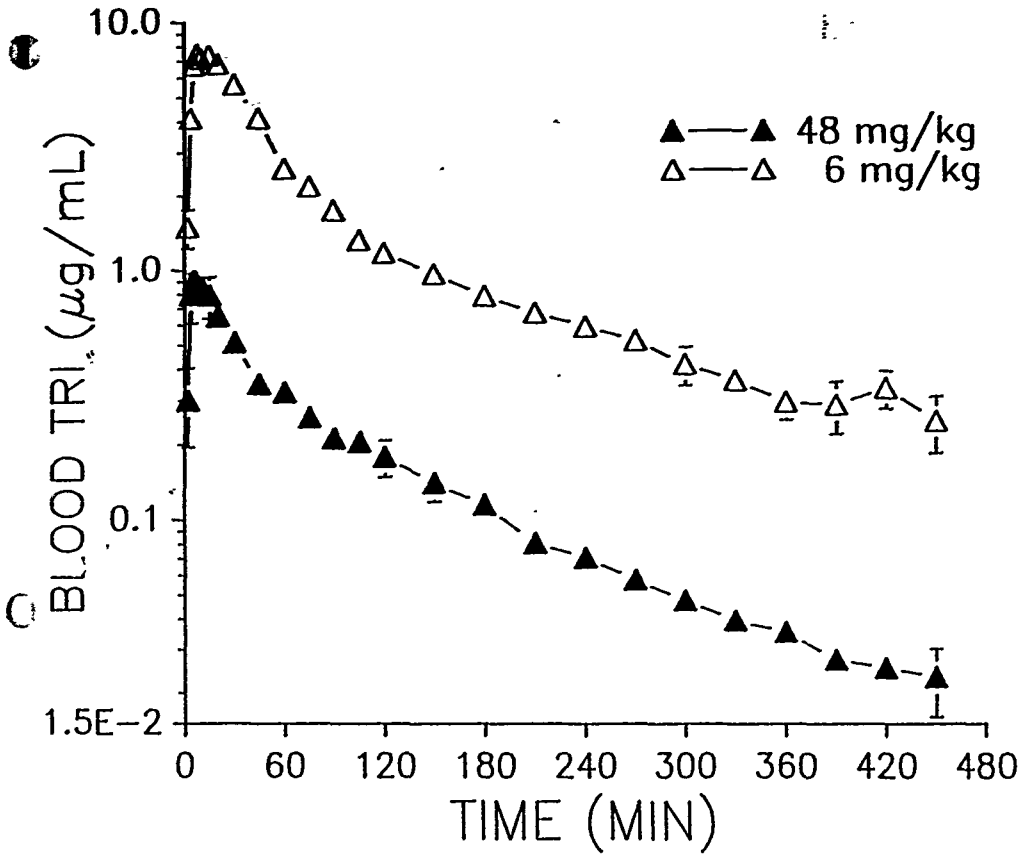
Values are the MEAN ± SE for 6 to 8 rats.

@ Concentration of TCE in arterial blood.

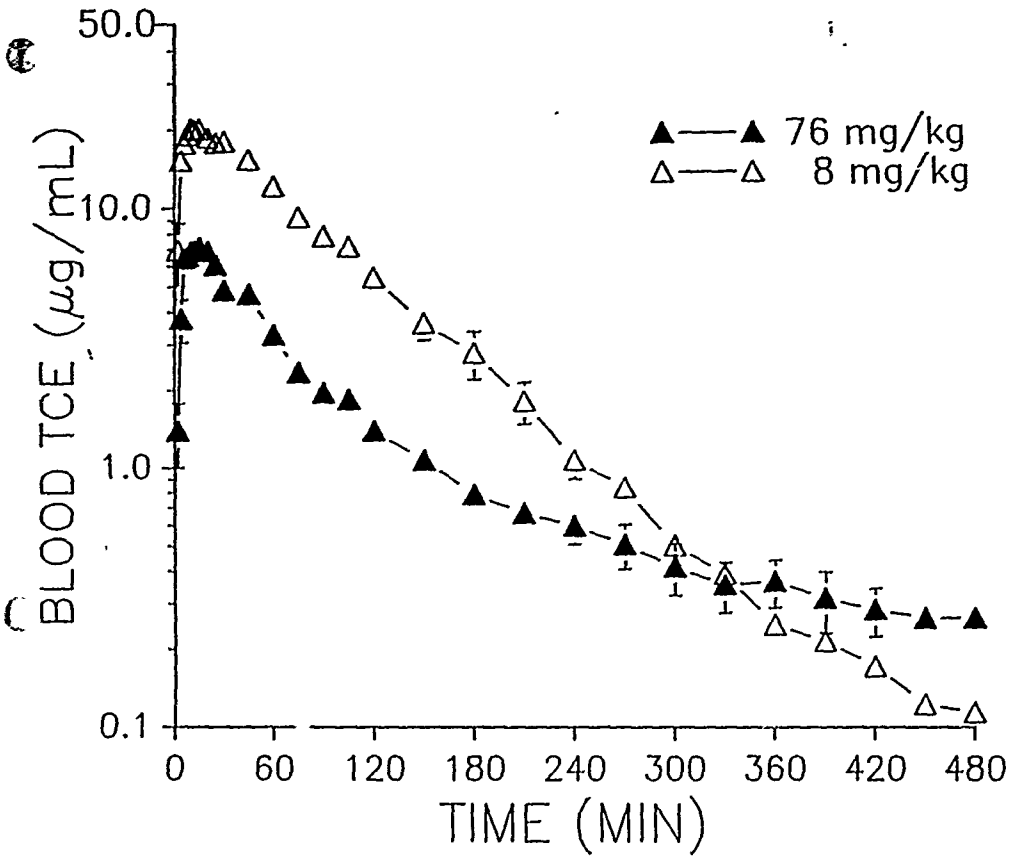


1,1-Dichloroethylene (DCE) concentrations in the blood of unanesthetized rats that have received a single oral bolus administration of 10 mg/kg or 30 mg/kg DCE in emulphor. Blood samples were taken from an indwelling carotid arterial cannula at 2 to 5 minute intervals during the rapid uptake phase and at 15 to 60 minute intervals during the elimination phase. Each value represents the mean ± SE for 6-8 rats.

Fig. D-1



1,1-Trichloroethane (TRI) concentrations in the blood of unanesthetized rats that have received a single oral bolus administration of 6 mg/kg or 48 mg/kg TRI in emulphor. Blood samples were taken from an indwelling carotid arterial cannula at 2 to 5 minute intervals during the rapid uptake phase and at 15 to 60 minute intervals thereafter. Each value represents the mean ± SE for 6-8 rats.



1,1,2-Trichloroethylene (TCE) concentrations in the blood of unanesthetized rats that have received a single oral bolus administration of 8 mg/kg or 76 mg/kg TCE in emulphor. Blood samples were taken from an indwelling carotid arterial cannula at 2 to 5 minute intervals during the rapid uptake phase and at 15 to 60 minute intervals thereafter. Each value represents the mean ± SE for 6-8 rats.

APPENDIX E

STUDIES OF THE PHARMACOKINETIC OF PCE

1) During and following inhalation exposures in rats

2) Following oral administration in rats

Parameters for the Physiological Pharmacokinetic Model for PER in the Rat

Parameter	Value
Alveolar Ventilation Rate (ml/min), V_a	115.3 (50 ppm exposure)
	101.4 (500 ppm exposure)
Inhaled Gas Concentration (mg/ml)	0.351 (50 ppm exposure)
	3.55 (500 ppm exposure)
Alveolar Mass Transfer Coefficient	500 ml/min
Blood Flows (ml/min)	
Cardiac output, Q_b	106.4
Fat, Q_f	9.4
Liver, Q_{li}	39.8
Muscle, Q_m	12.8
Richly Perfused, Q_r	44.4
Tissue Volumes (ml)	
Alveolar	2.0
Blood	25.4
Fat	30.5
Liver	13.6
Lung	3.97
Muscle	248.0
Richly Perfused	15.2
Partition Coefficients	
Lungs:Air	70.3
Fat:Blood	108.99
Liver:Blood	3.72
Muscle:Blood	1.058
Richly Perfused: Blood	3.72
Metabolism Constants	
Vmax (µg/min)	5.86
Km (µg/ml)	2.938

Alveolar ventilation rates and inhaled concentrations were directly measured in the laboratory. Compartmental volumes and organ blood flows were obtained from Ramsey and Andersen (Toxicol. Appl. Pharmacol. 73:159-175,1984) and scaled to 340 g , the mean bw of rats in the present study. Partition coefficients and metabolism constants were utilized from Chen and Blancato (Pharmacokinetics in Risk Assessment, Drinking Water and Health Vol. 8:369-390, 1987). The alveolar mass transfer coefficient was based on the alveolar permeability-area product for methylene chloride of Angelo and Pritchard (Pharmacokinetics in Risk Assessment, Drinking Water and Health Vol.8:254-264,1987).

INHALATION EXPOSURE SYSTEM

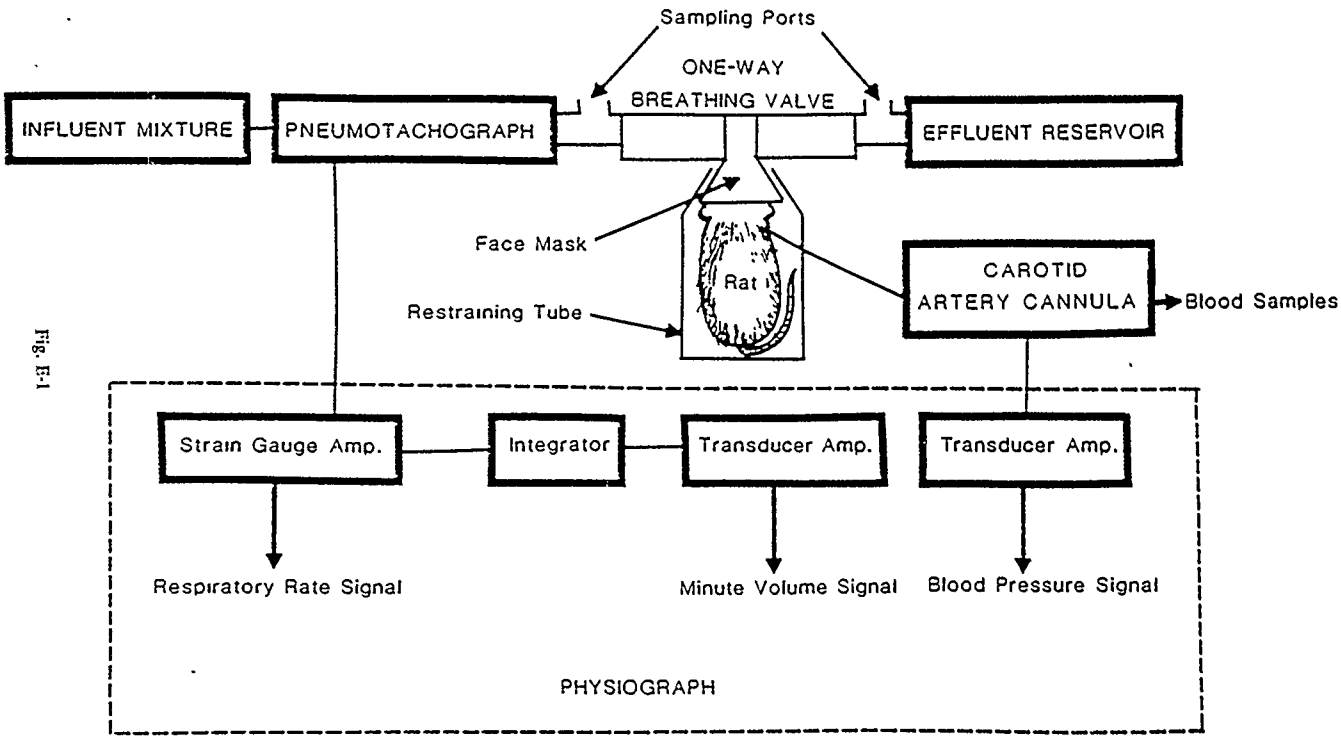


Fig. E-1

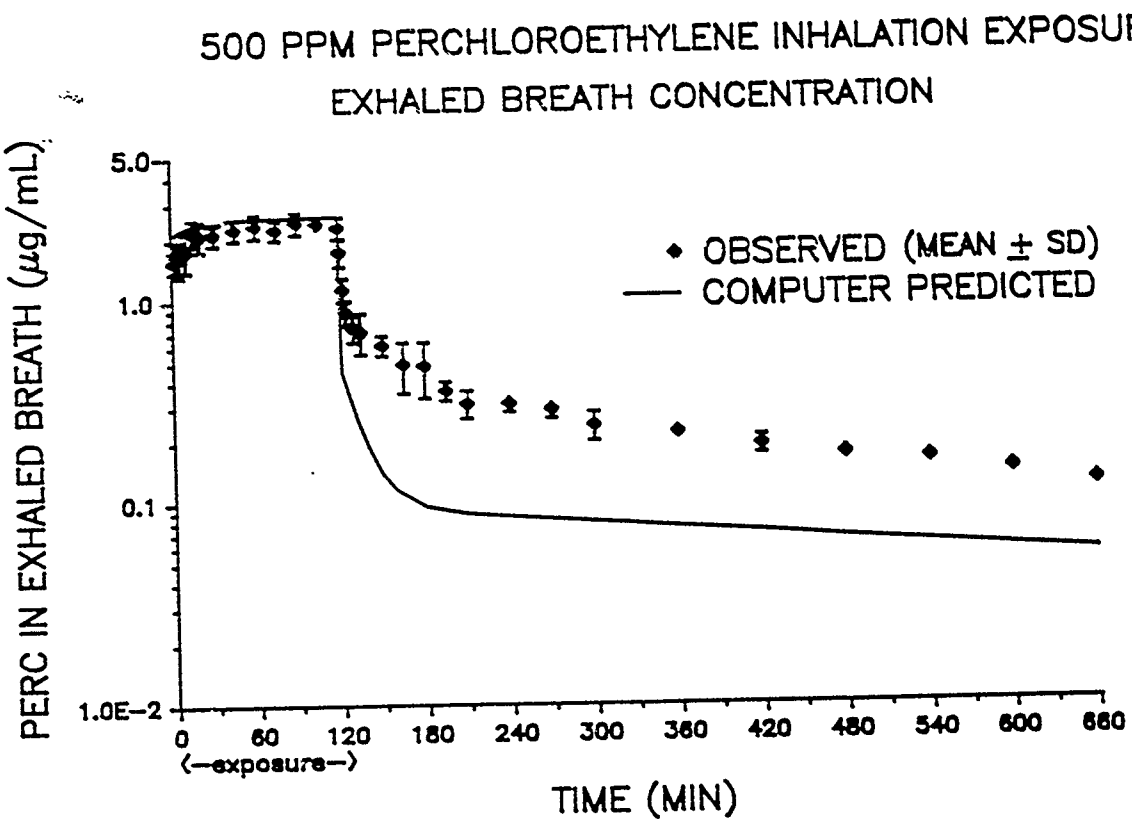

500 PPM PERCHLOROETHYLENE INHALATION EXPOSURE
EXHALED BREATH CONCENTRATION
PERC IN EXHALED BREATH (μg/mL)
5.0
1.0
0.1
1.0E-2
OBSERVED (MEAN ± SD)
COMPUTER PREDICTED
0
60
120
180
240
300
360
420
480
540
600
660
<—exposure—>
TIME (MIN)

Fig. E-2

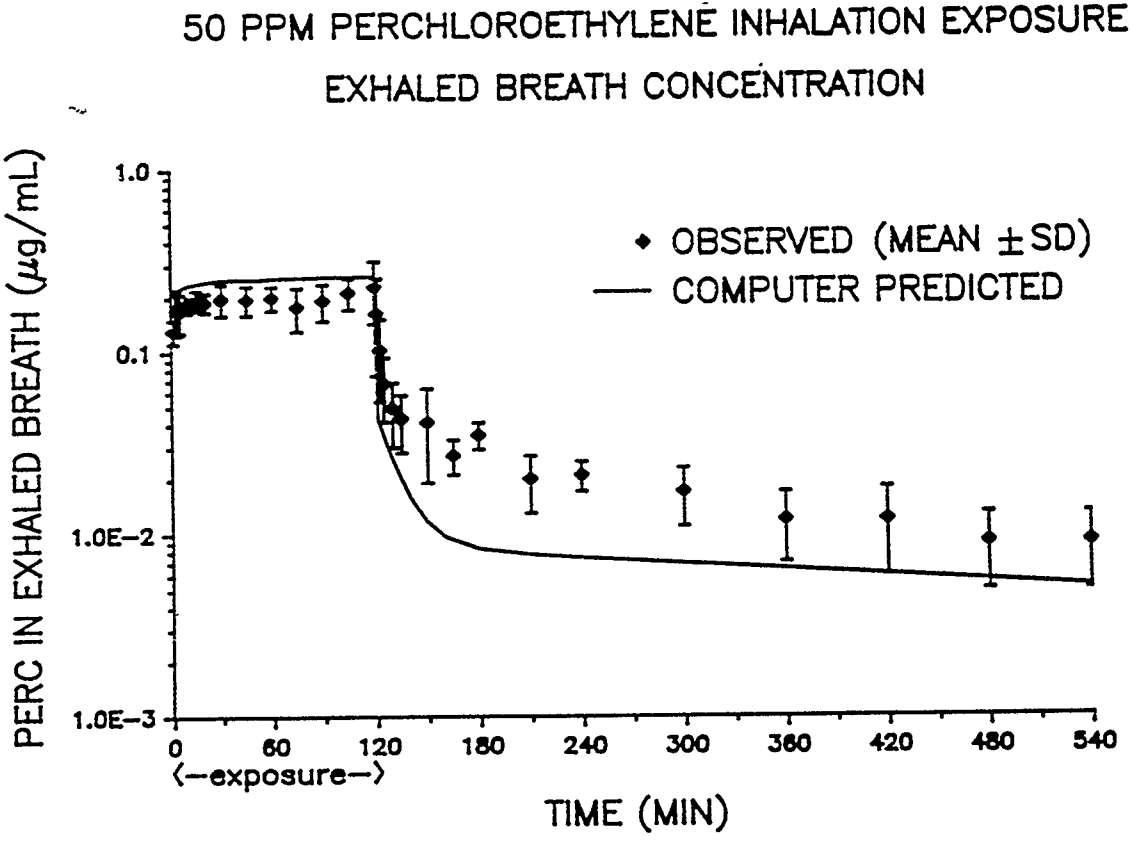

50 PPM PERCHLOROETHYLENE INHALATION EXPOSURE
EXHALED BREATH CONCENTRATION
PERC IN EXHALED BREATH (µg/mL)
1.0
0.1
1.0E-2
1.0E-3
◆ OBSERVED (MEAN ± SD)
COMPUTER PREDICTED
0
60
120
180
240
300
360
420
480
540
<—exposure—>
TIME (MIN)

Fig. E-3

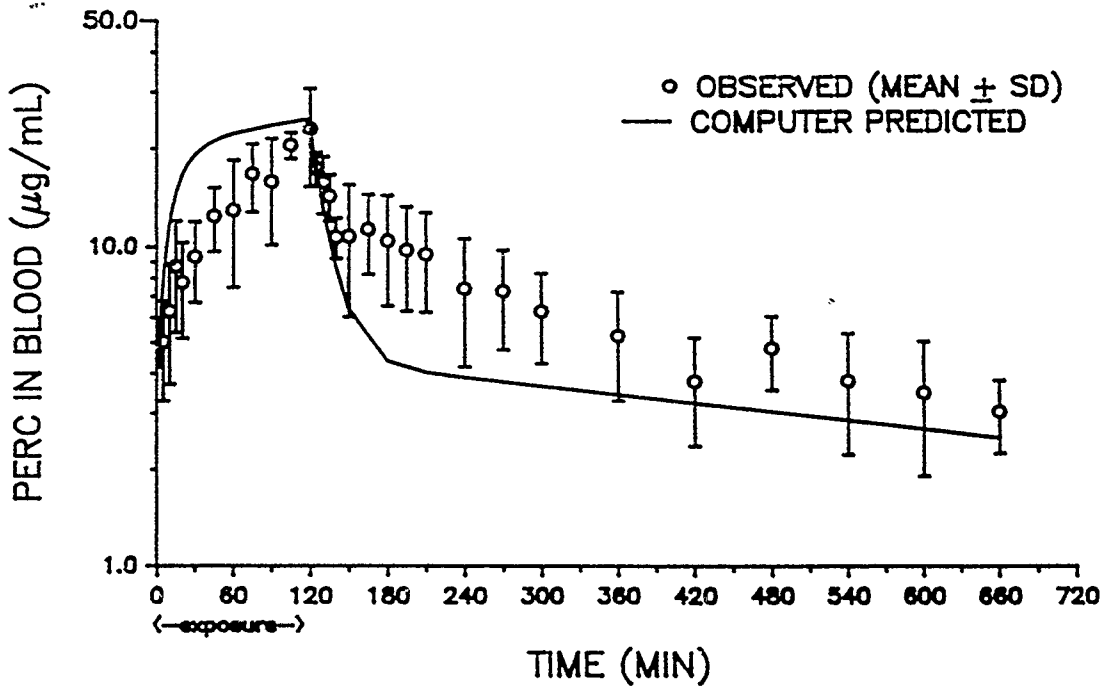

500 PPM PERCHLOROETHYLENE INHALATION EXPOSURE
ARTERIAL BLOOD CONCENTRATION
PERC IN BLOOD (μg/mL)
50.0
10.0
1.0
o OBSERVED (MEAN ± SD)
COMPUTER PREDICTED
0
60
120
180
240
300
360
420
480
540
600
660
720
<—exposure—>
TIME (MIN)

Fig. E-4

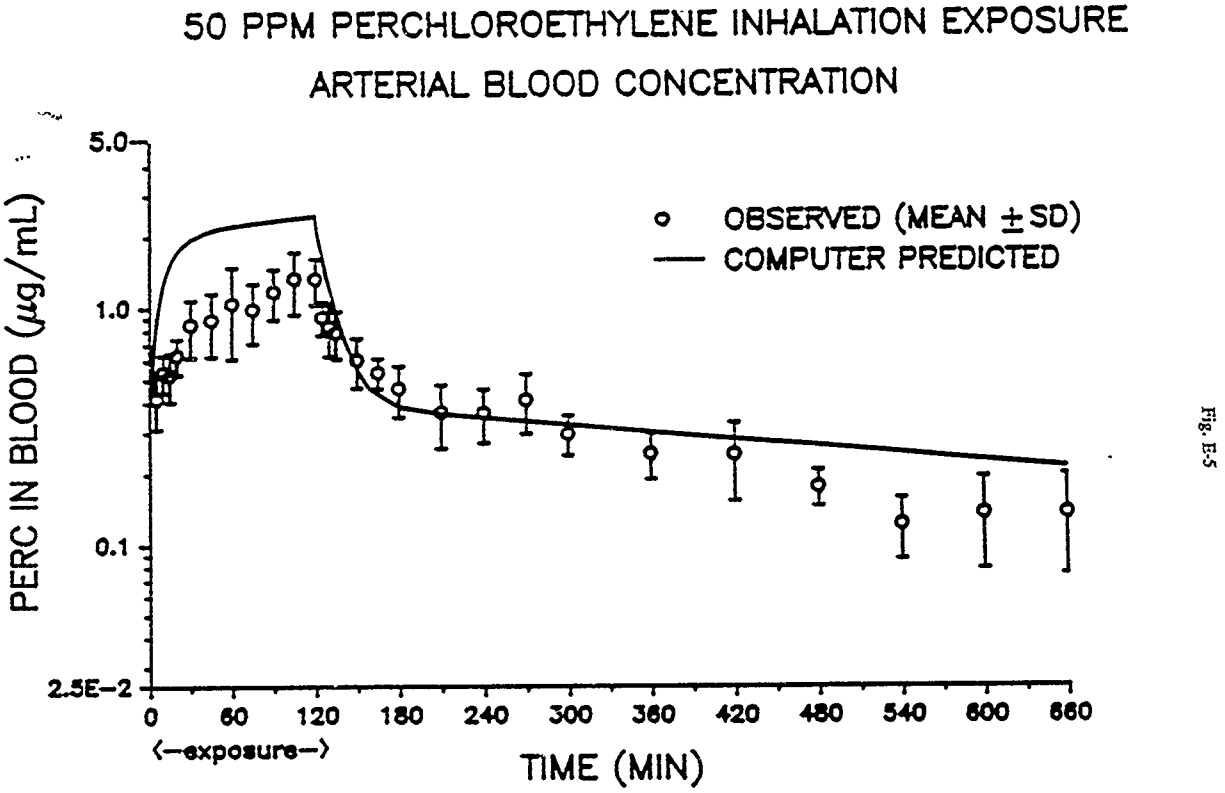

50 PPM PERCHLOROETHYLENE INHALATION EXPOSURE
ARTERIAL BLOOD CONCENTRATION
PERC IN BLOOD (μg/mL)
5.0
1.0
0.1
2.5E−2
OBSERVED (MEAN ± SD)
COMPUTER PREDICTED
0
60
120
180
240
300
360
420
480
540
600
660
〈—exposure—〉
TIME (MIN)

Fig. E-5

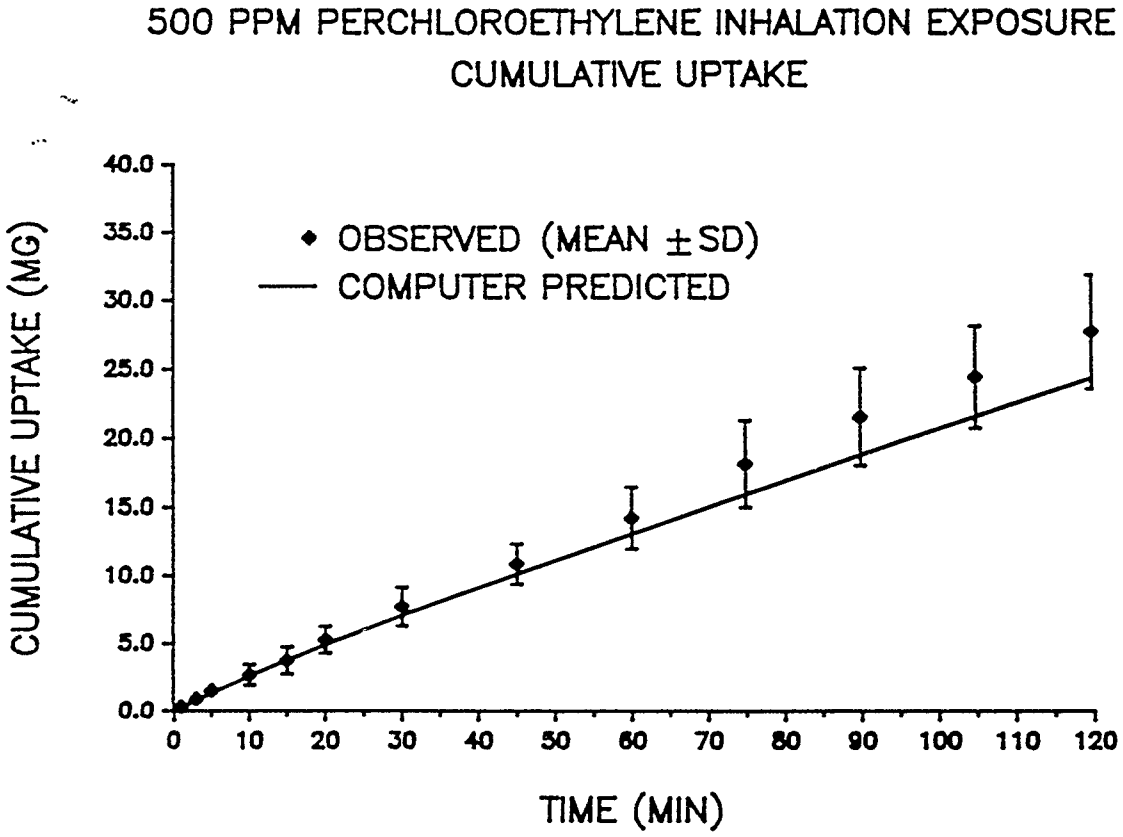

500 PPM PERCHLOROETHYLENE INHALATION EXPOSURE
CUMULATIVE UPTAKE
CUMULATIVE UPTAKE (MG)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
OBSERVED (MEAN ± SD)
COMPUTER PREDICTED
0
10
20
30
40
50
60
70
80
90
100
110
120
TIME (MIN)

Fig. E-6

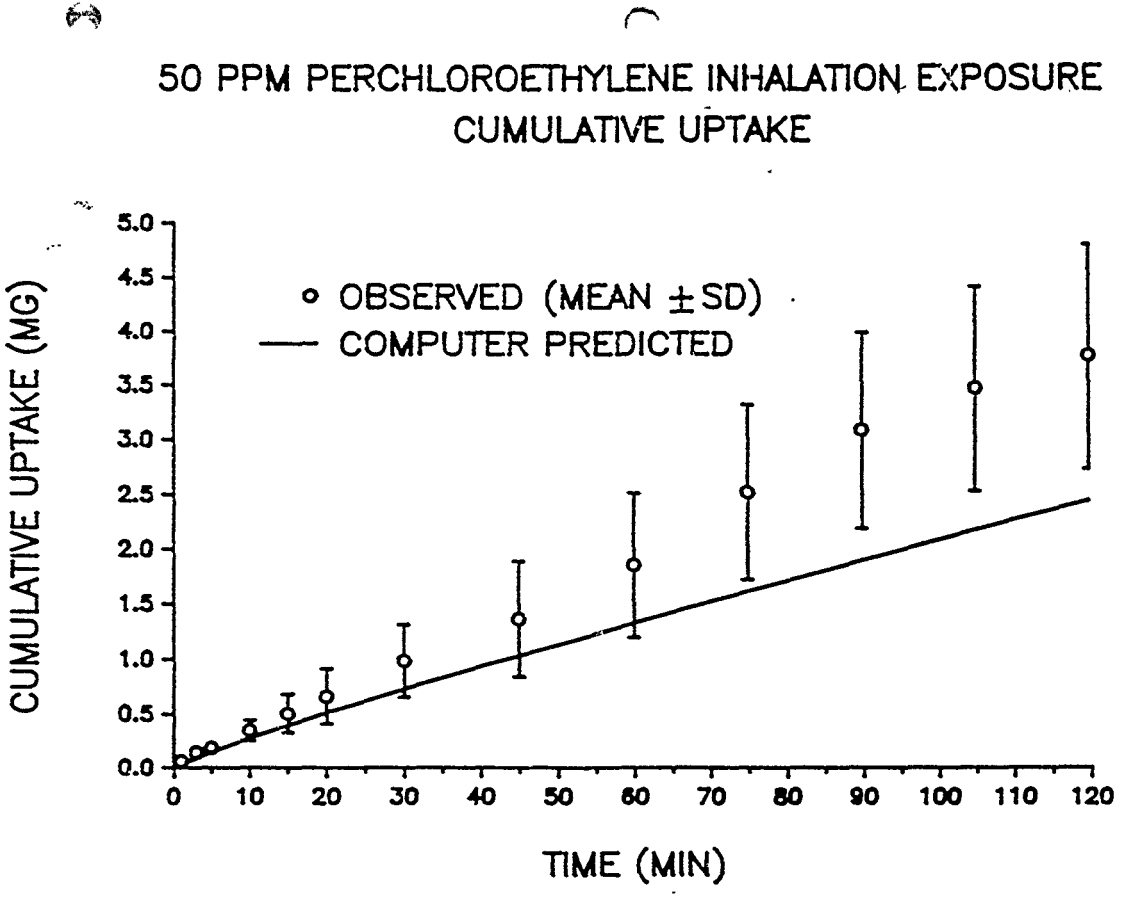

50 PPM PERCHLOROETHYLENE INHALATION EXPOSURE
CUMULATIVE UPTAKE
CUMULATIVE UPTAKE (MG)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
OBSERVED (MEAN ± SD)
COMPUTER PREDICTED
0
10
20
30
40
50
60
70
80
90
100
110
120
TIME (MIN)

Fig. B-7

PERCHLOROETHYLENE

ORAL BOLUS

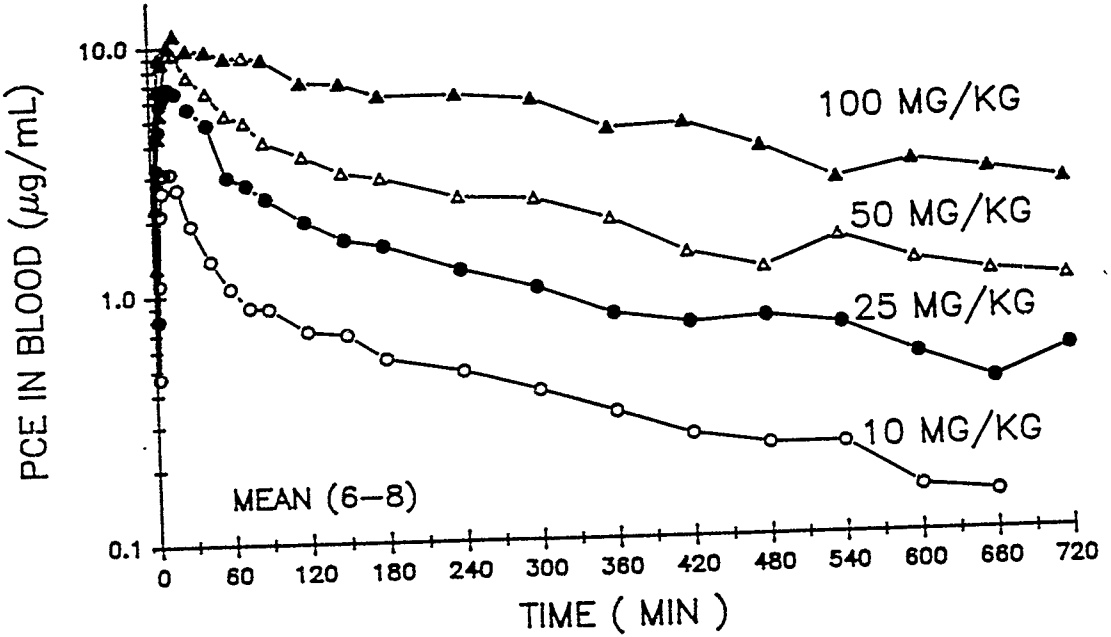


Fig. E-8

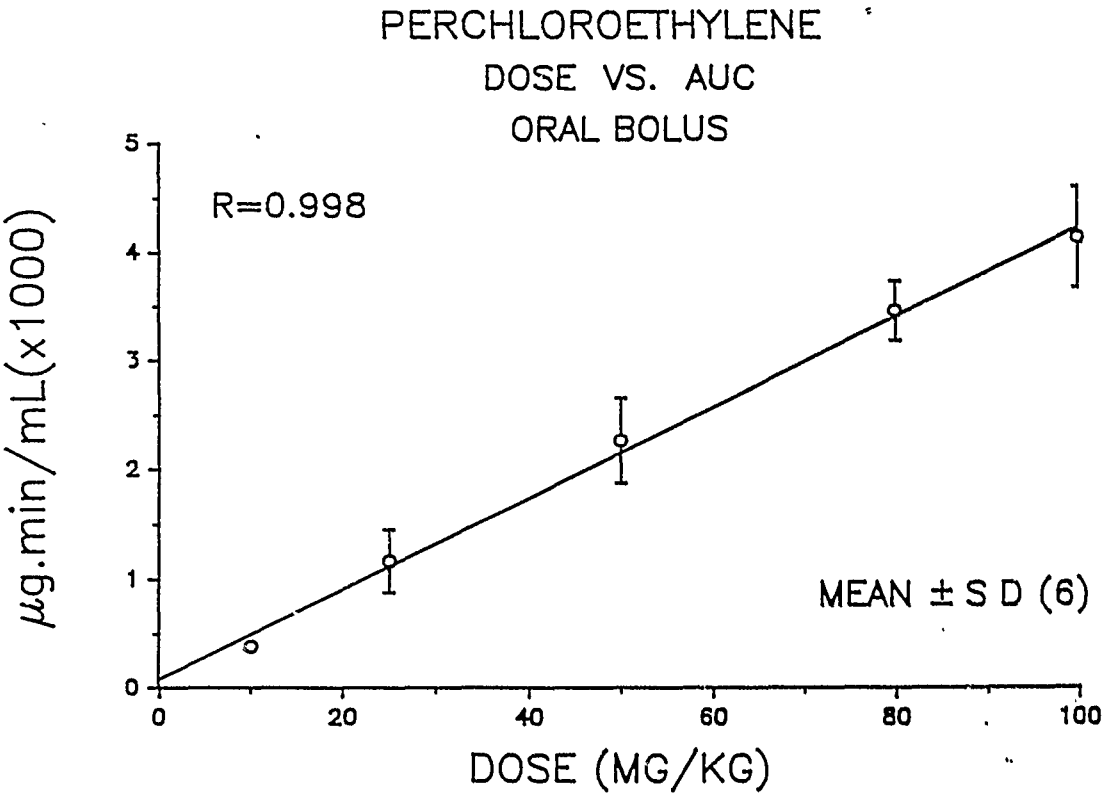

PERCHLOROETHYLENE
DOSE VS. AUC
ORAL BOLUS
R=0.998
MEAN ± S D (6)
μg.min/mL(x1000)
DOSE (MG/KG)
0
1
2
3
4
5
0
20
40
60
80
100

Fig. E-9

APPENDIX F

MANUSCRIPT NOW BEING SUBMITTED TO THE
JOURNAL OF ENVIRONMENTAL PATHOLOGY, TOXICOLOGY, AND ONCOLOGY

Chen, X.H., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. "Determination of volatile short-chain aliphatic halocarbons in animal tissues." Now being submitted to the Journal of Environmental Pathology, Toxicology, and Oncology (1991).

DETERMINATION OF VOLATILE SHORT-CHAIN ALIPHATIC HALOCARBONS IN ANIMAL TISSUES

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ABSTRACT

Characterization of the uptake, distribution and elimination of short-chain aliphatic organic halocarbons requires a reliable measurement technique for determining tissue concentrations. A head-space gas chromatographic method was developed to measure halocarbon concentrations in liver, kidney, brain, heart, lung, muscle, fat and blood. The method was evaluated using perchloroethylene (PER), 1,1,1-trichloroethane (TRI), tetrachloroethane (TET), and trichloroethylene (TCE). Approximately 1 gram of each tissue was injected with PER, TET, TCE, or TRI to yield a theoretical concentration of 4 ug halocarbon/gram tissue. Two homogenization procedures were evaluated: tissues were homogenized in saline, followed by isooctane extraction; tissues were homogenized in isooctane. Comparison of these two approaches for PER demonstrated a significantly higher percent recovery with the isooctane homogenization for most tissues. It was also observed that the aliquot volume of the tissue homogenate plays an important role in the headspace technique, in that greater than 30 μl aliquots could not be used. Percent recoveries ranged from 80-100 % for the seven tissues and blood for each of the four test chemicals.

Abbreviated title (running head). Determination of halocarbons in tissues

INTRODUCTION

Short-chain aliphatic halocarbons are a class of volatile organic compounds (VOCs) of increasing interest due to their widespread occurrence as environmental contaminants. Exposure to halocarbons can result in toxic injury in a number of organs in animals and humans. Central nervous system (CNS) dysfunction results from overexposure to most halocarbons[1,2], and this effect can be directly correlated with the tissue concentration of the halocarbon in the brain[3,4]. Significant liver and kidney toxicity can be caused by some halocarbons[5,6], while select agents in this chemical class are known carcinogens in various organ systems[7,8].

Pharmacokinetic studies of these chemicals are needed in order to evaluate the uptake, disposition, and elimination of these contaminants after exposure. There is a particular need for the measurement of halocarbons in the various tissues where the agents accumulate and where toxic effects are known to occur. Halocarbon analyses have been conducted in blood, exhaled breath, and tissues[9-15]. The tissue measurements conducted in these studies, however, employed radiolabelled halocarbons. Of course, these measurements do not delineate between the parent compound to which the animal was exposed and potential metabolites formed in the body following exposure. There is therefore a need for a reliable and sensitive analytical procedure for directly determining the concentrations of short-chain aliphatic halocarbons in the tissues of exposed animals. A head-space gas chromatographic technique is described for analyzing short-chain aliphatic halocarbons in animal tissues. Four representative chemicals of this class of VOCs are employed, in order to demonstrate the utility of the technique on chemicals with a variety of physicochemical properties.

MATERIAL AND METHODS

Apparatus

1. Gas chromatograph. Model 300 (Perkin-Elmer Co. Norwalk, CT)
   Model 5890 (Hewlett-Packard Co. Avondale, PA)
2. Stainless-steel column. 6'x1/8" FFAP (Alltech Associates, Deerfield, IL)
   OV-17 (Alltech Associates, Deerfield, IL)
3. Centrifuge. RC2-B (Sorvall, Norwalk, CT)
   IEC Centra-7r (International Equipment Company, Needham, MA)
4. Homogenizer. Janke & Kunkel, Ika-Werk, Ultra-Turrax SDT (Tekmar Company, Cincinnati, OH)
5. Touch Mixer. Model 231 (OH)
7. Rubber septa (PTFE Coated Butyl Rubber), 8 ml vials, caps (Perkin-Elmer Co., Norwalk, CT)

Test Chemicals

1. Perchloroethylene (PER). 99% purity (Aldrich Chemical Company Inc. Milwaukee, WIS)
2. 1,1,2,2,-tetrachloroethane (TET). 97% purity (Aldrich Company Inc. Milwaukee, WIS)
3. 1,1,1,-trichloroethane (TRI). (J.T.Baker Chemical Co. Phillipsburg, NJ)
4. Trichloroethylene (TCE). (J.T.Baker Chemical Co. Phillipsburg, NJ)
5. Isooctane. (J.T.Baker Chemical Co., Phillipsburg, NJ)
6. Ether,Anhydrous. (J.T.Baker Chemical Co. Phillipsburg, NJ)

Test Animal

Male-Sprague-Dawley rats. 325-375 grams (Charles River Laboratories, Raleigh, NC)

Tissue Homogenization and Extraction Procedure

Animals (n=8) were anesthetized with ether. One ml blood samples were withdrawn by closed cardiac puncture. From 0.5 to 1 gram each of liver, kidney, brain, heart, lung, perirenal fat, and muscle were removed and placed on ice. Four ul of PER, TET, TCE, and TRI were carefully injected into each tissue by using a Hamilton microsyringe, so that a theoretical concentration of 4 $\mu g/g$ was obtained. Two homogenization approaches were evaluated using PER. In the saline homogenization approach the tissues were immediately transferred after halocarbon injection to previously chilled 20 ml glass vials containing 4 ml saline. The tissues were then homogenized in saline. In the isooctane homogenization approach, the tissues were immediately transferred after halocarbon injection to previously chilled 20 ml glass vials containing 2 ml saline and 8 ml isooctane. For both approaches, the tissue homogenizer was used to homogenize the samples Samples were maintained on an ice bath at all times, including the homogenization period. For TCE, TRI, and TET the approach employing only isooctane homogenization was used. Each tissue was homogenized for an established time interval, specific to that tissue and minimized to lower volatilization of the halocarbon during the procedure. Brain, liver, and fat were the most easily homogenized, requiring only 3-4 sec. Kidney, lung and heart required 5-8 sec of homogenization each. Muscle was the most difficult, requiring 20 seconds of homogenization. All samples were vortexed for 30 seconds following homogenization and centrifuged at 2700 rpm for 10 min at 4°C.

Homogenate Aliquot Volume

In order to study the effect of tissue homogenate volume on the linearity of the concentration measurements, various aliquot volumes were employed in transferring the tissue homogenate to the gas chromatography vials. A known quantity of PER was injected into blood and the test tissues as described previously. From 5 to 100 μl aliquots of the blood samples and homogenates of the 7 tissues were withdrawn with a pipet and transferred to 8 ml headspace vials. These vials were capped immediately with septa and a spring washer. Each sample vial was then placed into the autosampler unit of the gas chromatograph with an electron capture detector.

Headspace Gas Chromatographic Technique

For all the experiments utilizing PER, the operating conditions were: headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; column packing, 10% FFAP; flow rate for Argon/Methane carrier gas, 60 ml/min. Operating conditions for TET were: headspace sampler temperature, 100°C; injection port temperature, 200°C; column temperature, 150°C; detector temperature, 400°C; column packing, 3% OV-17; flow rate for Argon/Methane carrier gas, 60 ml/min. Operating conditions for TRI & TCE were: headspace sampler temperature, 55°C; injection port temperature 150°C; column temperature, 60°C, detector temperature, 400°C; column packing, 3% SP 1000. Except for the homogenate aliquot volume study, all measurements were made using a 20 μl aliquot of the tissue homogenate in the 8 ml headspace vials.

Standard solutions were made and assayed by diluting a specified amount of pure PER, TET, TCE, and TRI in isooctane. Standard curves for each chemical were then generated each day that measurements were conducted.

Statistics

Comparisons between the two homogenization procedures on the percent recovery of PER in seven rat tissues and in blood were made using the Student's t Test. Values were considered significantly different at $p < 0.05$.

RESULTS

The results of the study on the effect of homogenate aliquot volume are shown for blood and 7 tissues injected with PER in Fig. 1-4. A very similar pattern was found in all tissues studied. Concentration values were linear with increasing aliquot volume only up to 25 μl (except heart, which was linear only up to 20 μl). At 50 or 100 μl aliquot volumes, the concentration of PER in tissue or blood did not increase over the values seen at lower aliquot volumes.

Comparison of the percent recovery obtained using the saline and isooctane homogenization approaches for PER analyses is given in Table 1. Percent recovery of PER in kidney, fat, lung, muscle, and brain were significantly higher for the isooctane homogenization relative to the saline homogenization. Differences between the two approaches for liver, heart, and blood were not statistically significant.

The isooctane homogenization approach was used to determine the percent recovery for PER, TET, TCE, and TRI (Table 2). Except for the percent recovery of PER in lung and muscle, the recovery of TET was higher in all tissues relative to the other three chemicals tested. Percent recovery of TCE was generally the lowest of the four test chemicals with no mean recovery values exceeding 88% for any tissue. Indeed, the lowest percent recovery for TET (fat) was greater than the highest percent recovery for TCE (muscle). The mean percent recoveries of PER, TET, and TRI in fat were very similar (within 2%), while TCE fat recovery

was the lowest of any tissue group or chemical tested. The range in values for percent recovery between the various tissues for each chemical was the smallest for TRI (less than 6%), and the largest for PER (17.8%). The tissue with the longest homogenization time, muscle, had the highest percent recovery of the tissue groups for PER and TCE. The tissues with the shortest homogenization times (liver, fat, and brain) did not appear to have lower percent recoveries than the other non-muscle tissues within each chemical group.

DISCUSSION AND CONCLUSIONS

A headspace gas chromatographic technique for determining the concentration of halocarbons in tissues was developed that is reproducible and technically feasible. A purge and trap technique was developed previously for measuring tissue levels of the halocarbons 1,1- and 1,2-dichloroethylene[16]. This technique involved thermal desorption of halocarbon from tissues within a purging device and trapping on a Tenax column, with subsequent heat desorption on a gas chromatograph. While that approach was sensitive and reproducible, it was significantly labor intensive and time consuming. The procedure presented in this paper was quite reproducible, while utilizing only a homogenization step and the relative ease and speed of the headspace gas chromatographic approach.

A critical technical factor in the analysis was a limitation on the aliquot volume that could be employed in the headspace vials. As standard curve measurements were no longer linear to concentrations above a 25μl aliquot volume, a 20 μl aliquot was selected for use in the method. In a comparison of the two homogenization techniques, there was a statistically higher percent recovery for most of the tissues using the isooctane homogenization relative to using saline. It is possible that employing the saline homogenization in addition to the

subsequent step of mixing with isooctane provided additional opportunity for volatilization loss relative to the single step of homogenization in isooctane.

The applicability of this approach for a variety of halocarbons was demonstrated by its use with four halocarbons of significant variation in physicochemical properties. As would be expected, the relative volatility of the test agent had an effect on the percent recovery by the method. The boiling temperatures of TRI, TCE, PER, and TET are 74, 86.7, 121, and 140.7°C, respectively[17]. The chemical with the highest boiling temperature, TET, also had the highest percent recovery in most of the tissues relative to the other chemicals tested. TET was therefore less likely to volatilize during the homogenization procedure than TRI, TCE, or PER. Except for PER recovery in lung and muscle, the other tissue recoveries did not appear to differ greatly between these latter three chemicals. These two tissues had among the longest homogenization times employed, so the lesser volatility of PER relative to TRI and TCE was probably a factor in the increased recovery seen here.

The technique for measuring halocarbons in tissues should have a number of useful applications. Most validations of the utility of physiologically-based pharmacokinetic (PBPK) models to accurately predict the uptake and disposition of halocarbons have utilized blood, exhaled breath, and metabolic parameters. For instance, in the published validation of a PBPK model for TRI[18] the only tissue data the authors had to use were levels of radioactivity measured in the liver and fat of mice at the termination of an inhalation exposure to ^{14}C-TRI[19]. Since these models are based on tissue compartments, this technique for directly measuring the test compound in each tissue will be of significant benefit in verifying the predictions of these models. Comparison of these direct determinations of the present compound in tissues with previous studies of

radiolabelled chemicals may provide valuable insight into the rate and magnitude of metabolite formation from these compounds. Correlation of the measurement of chemical in target organs with the subsequent toxicity will also be useful in establishing dose response relationships for halocarbons at the organ level.

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19. Schumann AM, Fox TR, and Watanabe PG. [^{14}C]Methylcchloroform (1,1,1-trichloroethane): Pharmacokinetics in rats and mice following inhalation exposure. Toxicol. Appl. Pharmacol. 1982; 62: 390-401.

Table 1

Effect of Homogenization Procedure on Percent Recovery of PER
(mean ± SE)

	Saline Homogenization	Isooctane Homogenization
Liver	95.5 ± 9.9	89.6 ± 3.1
Kidney	69.0 ± 4.9***	86.7 ± 1.4***
Fat	73.8 ± 4.9*	88.2 ± 2.7*
Heart	75.8 ± 10.3	81.2 ± 1.2
Lung	78.8 ± 7.1**	99.1 ± 2.3**
Muscle	80.3 ± 6.3*	98.5 ± 2.9*
Brain	72.3 ± 5.7**	88.6 ± 2.0**
Blood	104.8 ± 5.5	95.4 ± 4.1

* Statistically significant difference between procedures at $p < 0.05$.
** Statistically significant difference between procedures at $p < 0.01$.
*** Statistically significant difference between procedures at $p < 0.001$.

Table 2

Percent Recovery for Aliphatic Halocarbons in Blood and Tissues
(mean ± SE)

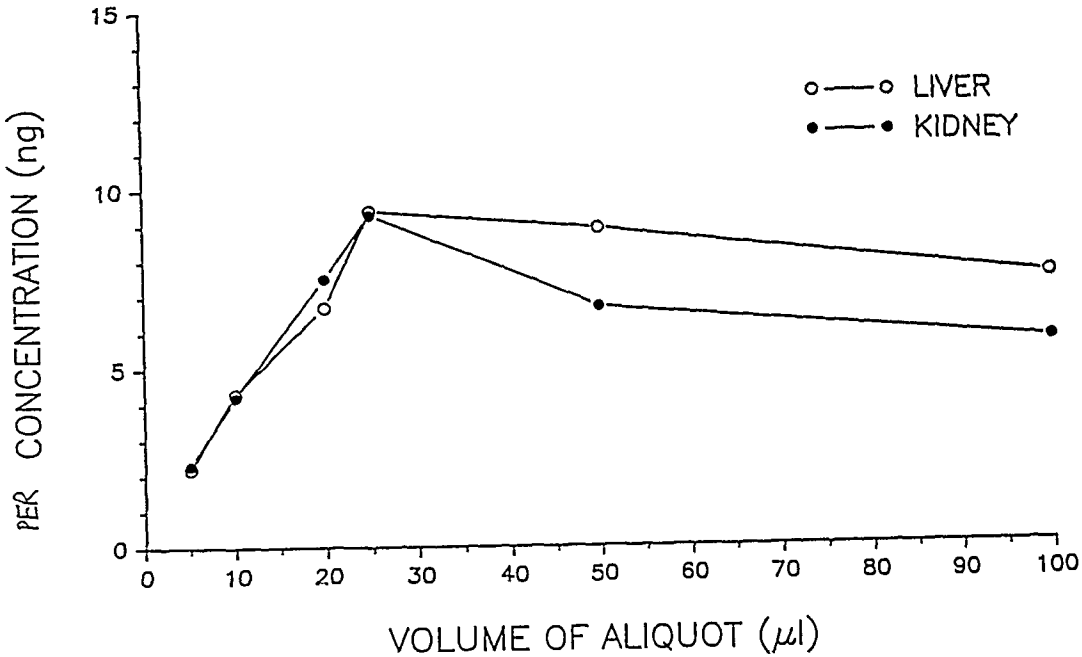
	PER	TET	TCE	TRI
Liver	89.6 ± 3.1	96.1 ± 2.1	86.0 ± 1.3	91.3 ± 4.0
Kidney	86.7 ± 1.4	97.9 ± 1.5	86.9 ± 1.6	88.4 ± 4.9
Fat	88.2 ± 2.7	89.9 ± 1.4	73.0 ± 1.5	88.7 ± 2.2
Heart	81.2 ± 1.2	98.1 ± 1.4	85.9 ± 3.5	89.6 ± 2.4
Lung	99.1 ± 2.3	96.1 ± 0.8	80.0 ± 0.7	89.7 ± 1.9
Muscle	98.5 ± 2.9	97.4 ± 1.1	87.9 ± 1.7	87.6 ± 4.9
Brain	88.6 ± 2.0	100.3 ± 3.1	80.7 ± 2.8	87.6 ± 4.6
Blood	95.4 ± 4.1	97.3 ± 2.1	85.9 ± 2.0	85.5 ± 3.7

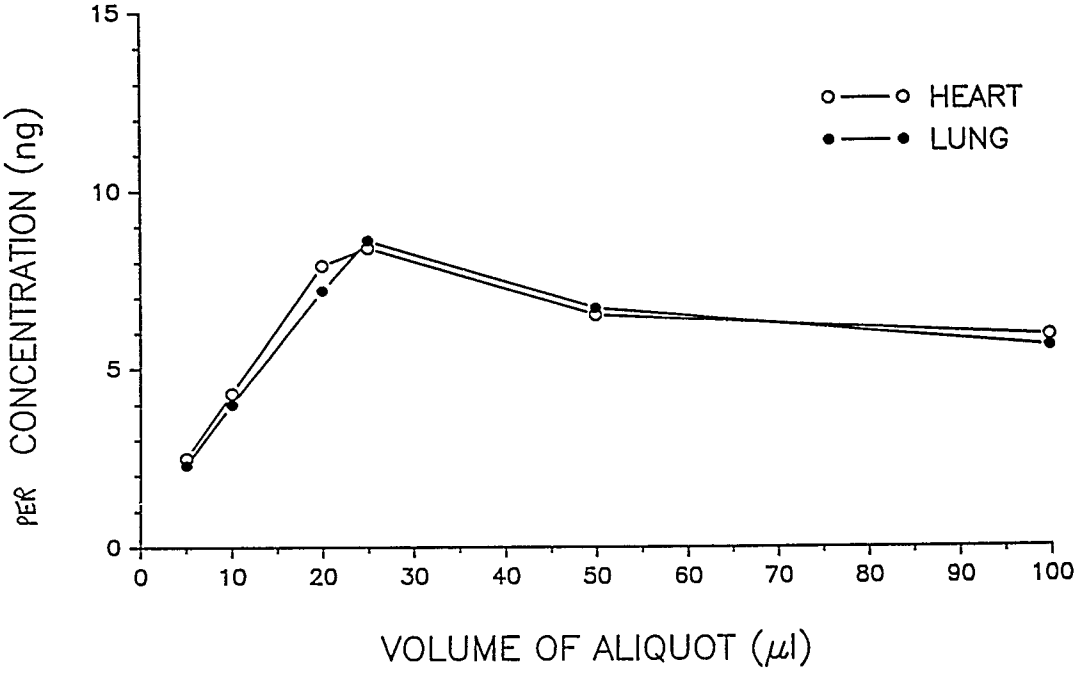
Fig. 1 Effect of aliquot volume on PER concentration linearity for liver and kidney tissue samples. Aliquot volumes of 5, 10, 20, 25, 50, and 100 μl were withdrawn from the organic phase of the tissue homogenate and analyzed.

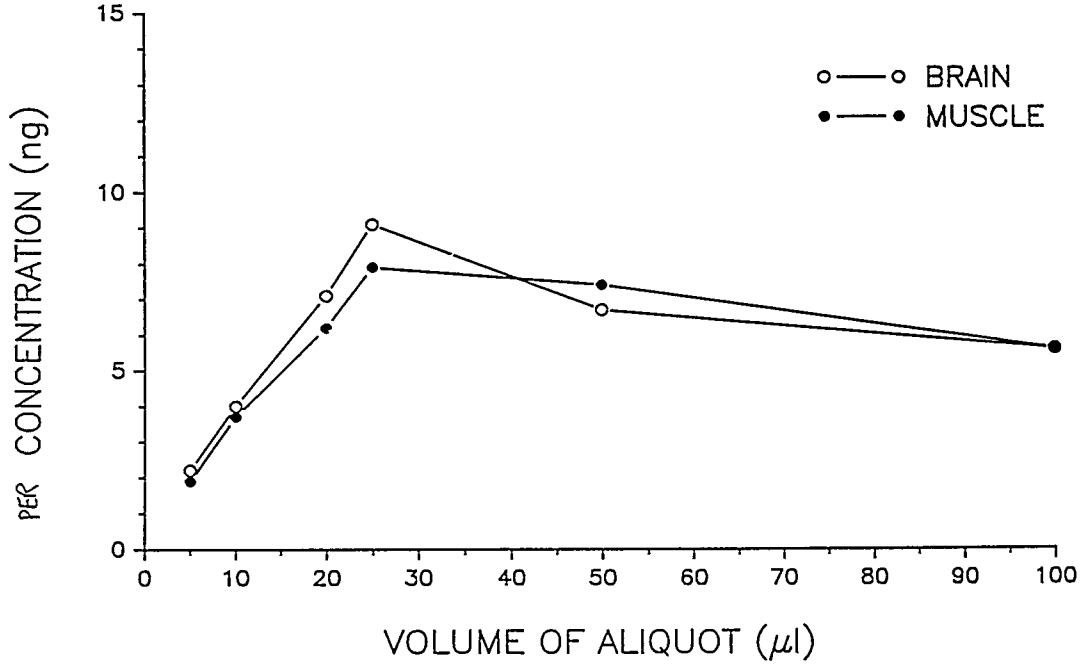
Fig. 2 Effect of aliquot volume on PER concentration linearity for heart and lung tissue samples. Aliquot volumes of 5, 10, 20, 25, 50, and 100 μl were withdrawn from the organic phase of the tissue homogenate and analyzed.

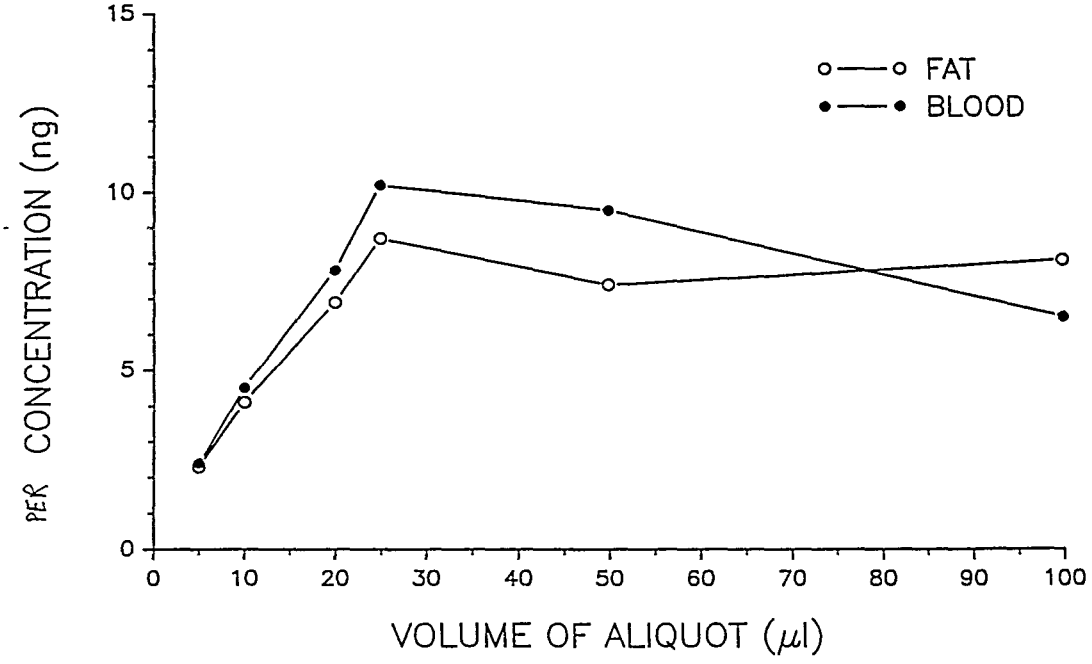
Fig. 3 Effect of aliquot volume on PER concentration linearity for brain and muscle tissue samples. Aliquot volumes of 5, 10, 20, 25, 50, and 100 μl were withdrawn from the organic phase of the tissue homogenate and analyzed.

Fig. 4 Effect of aliquot volume on PER concentration linearity for fat and blood tissue samples. Aliquot volumes of 5, 10, 20, 25, 50, and 100 μl were withdrawn from the organic phase of the tissue homogenate and analyzed.


15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
PER CONCENTRATION (ng)
VOLUME OF ALIQUOT (μl)
LIVER
KIDNEY


15
10
5
0
PER CONCENTRATION (ng)
HEART
LUNG
0
10
20
30
40
50
60
70
80
90
100
VOLUME OF ALIQUOT (μl)


15
10
5
0
PER CONCENTRATION (ng)
0
10
20
30
40
50
60
70
80
90
100
VOLUME OF ALIQUOT (μl)
BRAIN
MUSCLE


15
10
5
0
PER CONCENTRATION (ng)
FAT
BLOOD
0
10
20
30
40
50
60
70
80
90
100
VOLUME OF ALIQUOT (μl)

APPENDIX G

STUDIES OF THE TISSUE UPTAKE, DISPOSITION, AND ELIMINATION OF PCE IN RATS

1) Following intraarterial administration

2) Following oral administration

PHARMACOKINETIC PARAMETERS
OBSERVED TISSUE CONCENTRATIONS

PER IA ADMINISTRATION (10mg/kg)

TISSUE	AUC (µg·min/g)	t½ (hrs)	C_{max} (µg/g)
LIVER	2367	6.6	25.57
KIDNEY	2191	6.6	22.55
FAT	66962	7.7	66.98
HEART	1411	7.1	15.26
LUNG	1181	7.7	10.70
MUSCLE	1658	7.3	9.01
BRAIN	2354	6.7	25.21
BLOOD	555	7.8	5.48

PER INHALATION EXPOSURE (500ppm/2hrs)

TISSUE	AUC (µg·min/g)	t½ (hrs)	C_{max} (µg/g)
LIVER	32796	7.7	155.60
KIDNEY	27123	7.5	109.37
FAT	1545750	9.6	1567.54
HEART	24264	5.5	109.67
LUNG	19697	7.0	98.38
MUSCLE	26130	7.0	91.13
BRAIN	34450	6.9	177.42
BLOOD	9062	5.6	47.13

Table G-1

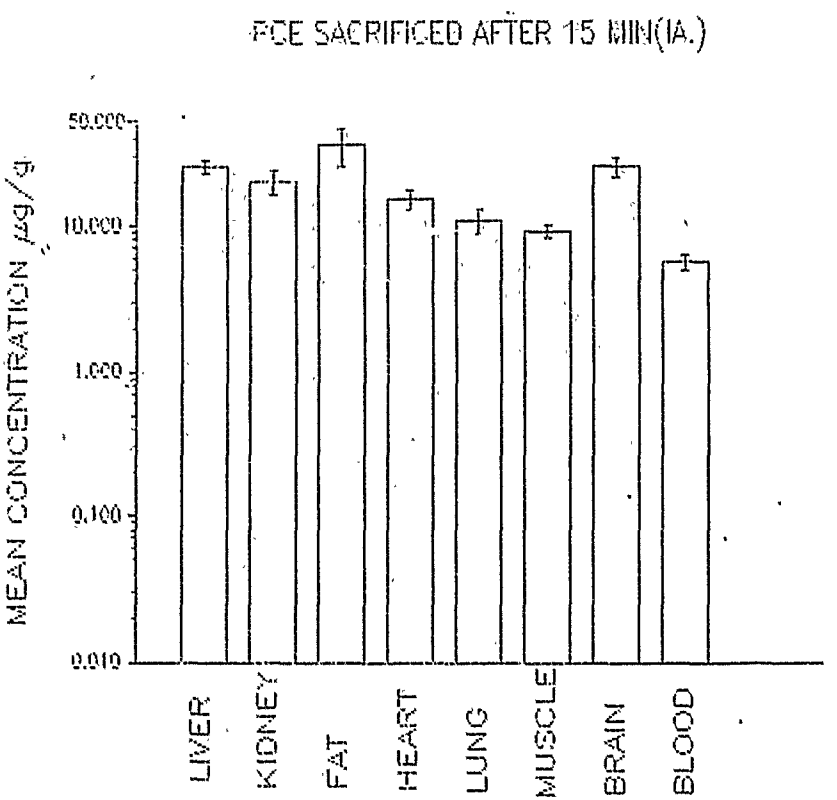

PCE SACRIFICED AFTER 15 MIN(IA.)
MEAN CONCENTRATION µg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-1

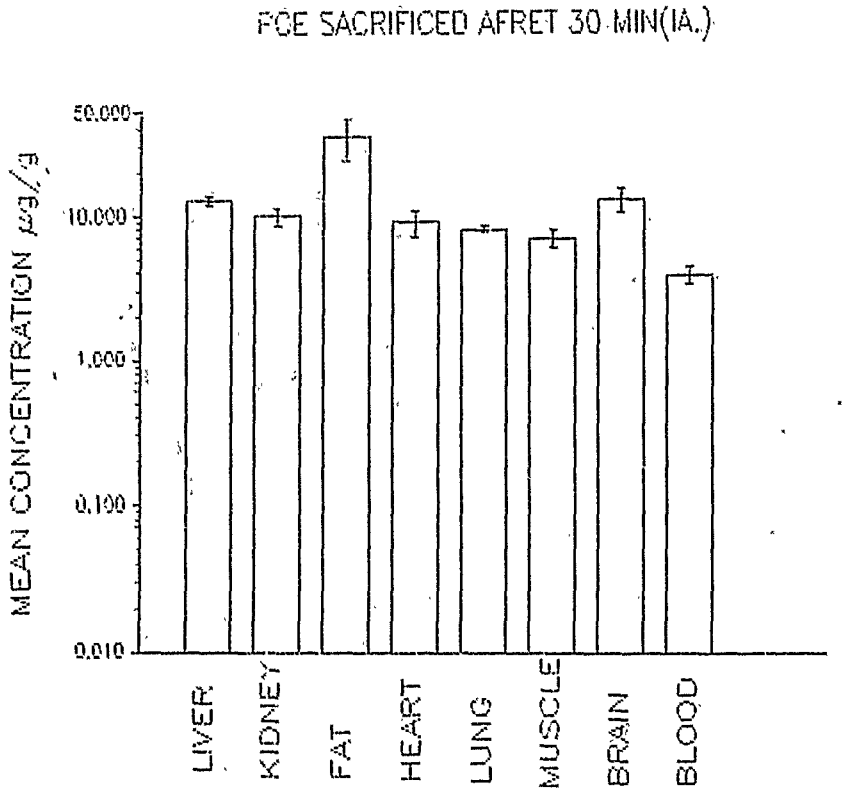

FCE SACRIFICED AFRET 30 MIN(IA.)
MEAN CONCENTRATION μg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-2

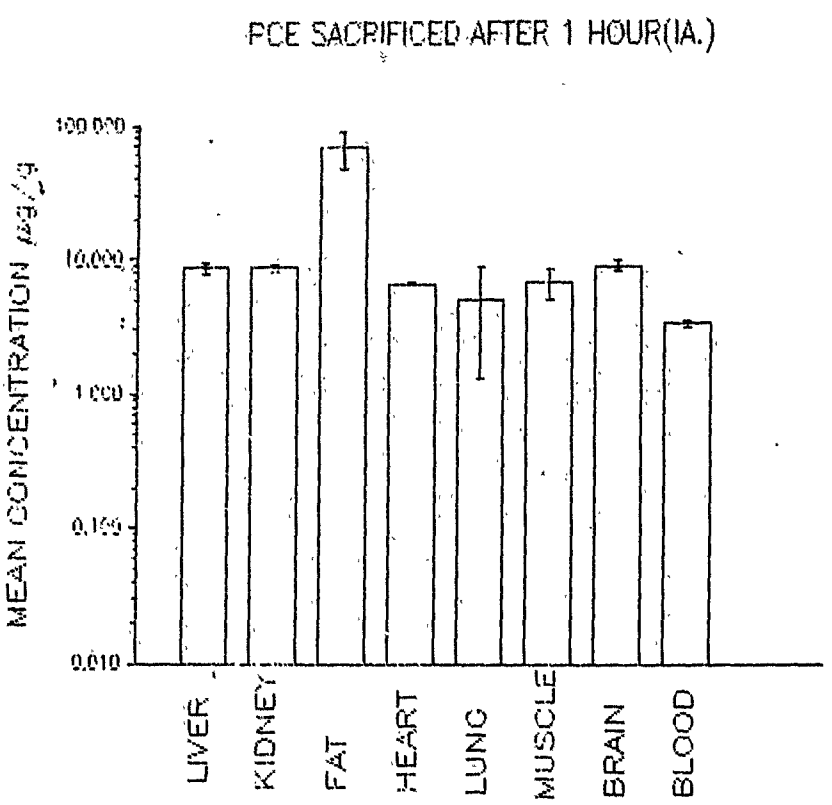

PCE SACRIFICED AFTER 1 HOUR(IA.)
MEAN CONCENTRATION µg/g
100 000
10,000
1 000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-3

PCE SACRIFICED AFTER 2 HOURS(IA.)
MEAN CONCENTRATION μg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-4

PCE SACRIFICED AFTER 4 HOURS(IA.)
MEAN CONCENTRATION µg/g
100.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-5

PCE SACRIFICED AFTER 6 HOURS(IA.)

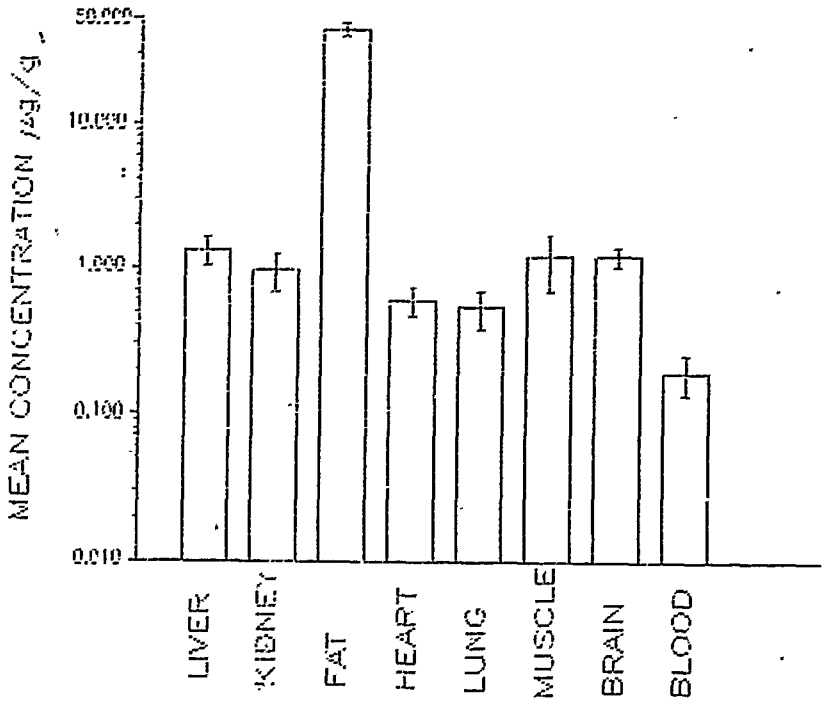


Fig. G-6

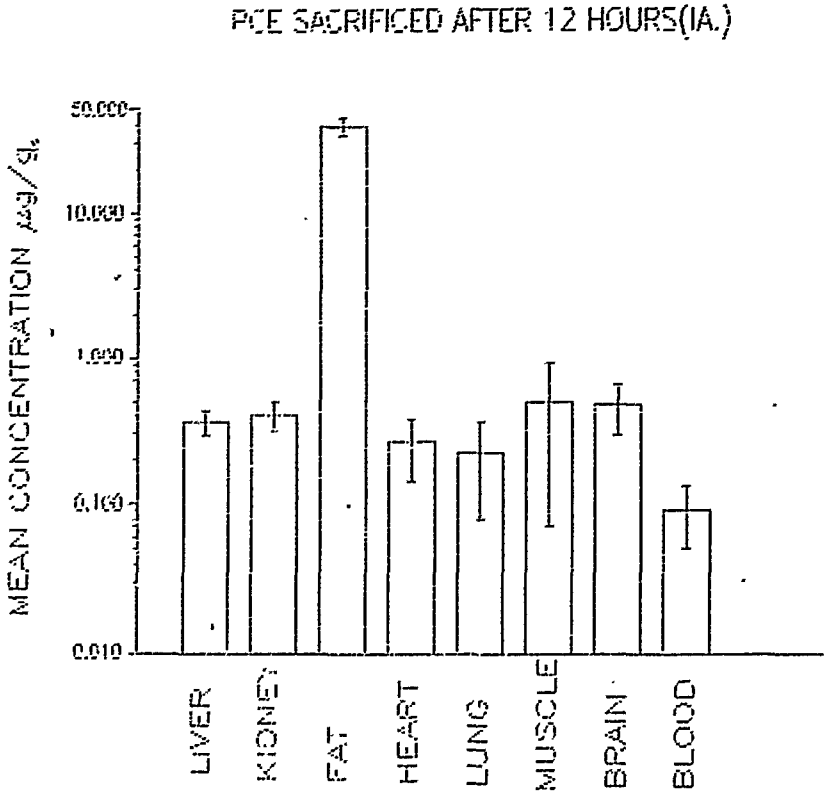

PCE SACRIFICED AFTER 12 HOURS(IA.)
MEAN CONCENTRATION µg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-7

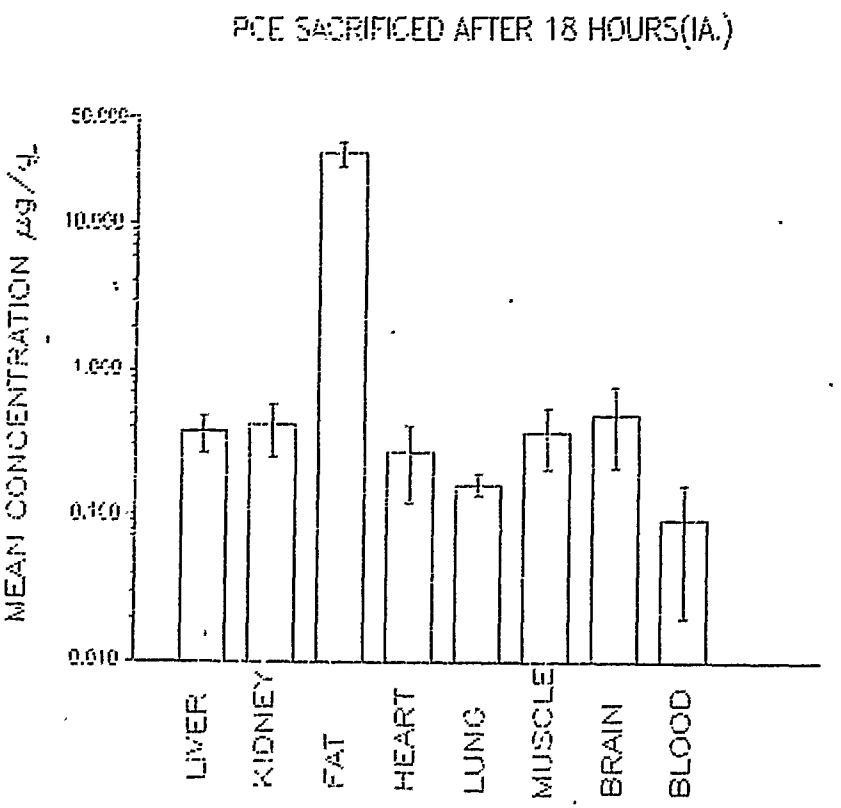

PCE SACRIFICED AFTER 18 HOURS(IA.)
MEAN CONCENTRATION µg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-8

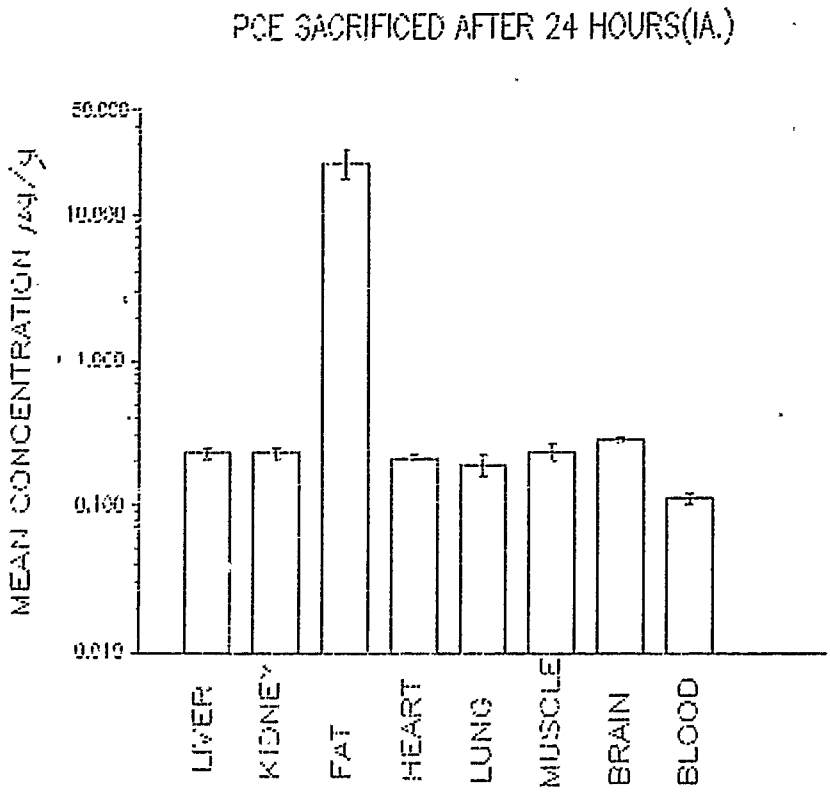

PCE SACRIFICED AFTER 24 HOURS(IA.)
MEAN CONCENTRATION
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-9

PCE SACRIFICED AFTER 48 HOURS(IA.)
MEAN CONCENTRATION µg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-10

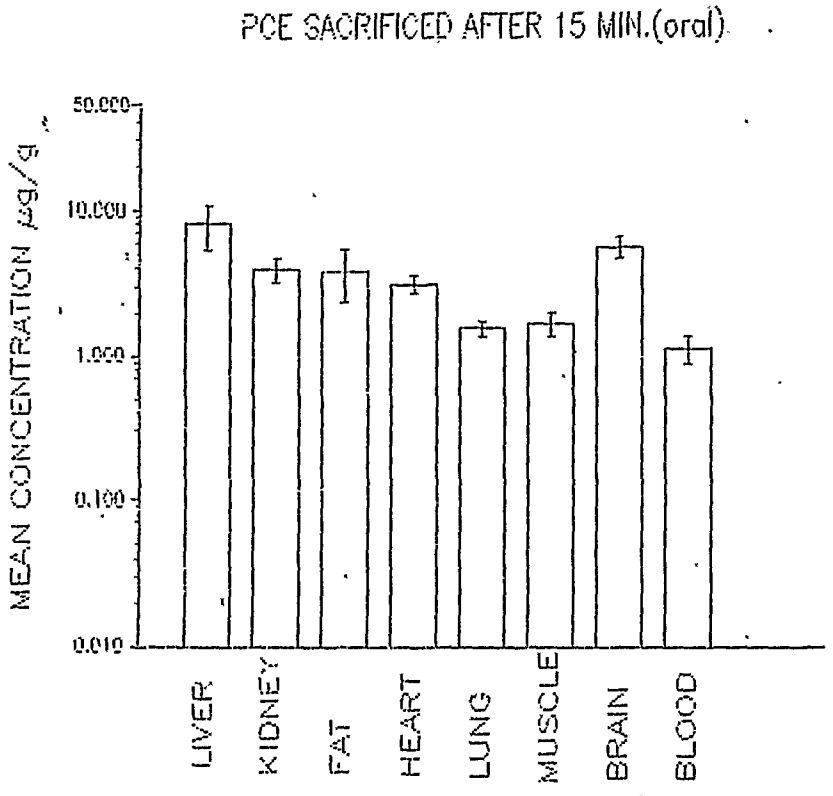

PCE SACRIFICED AFTER 15 MIN.(oral)
MEAN CONCENTRATION μg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-11

PCE SACRIFICED AFTER 30 MIN.(oral)
MEAN CONCENTRATION μg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-12

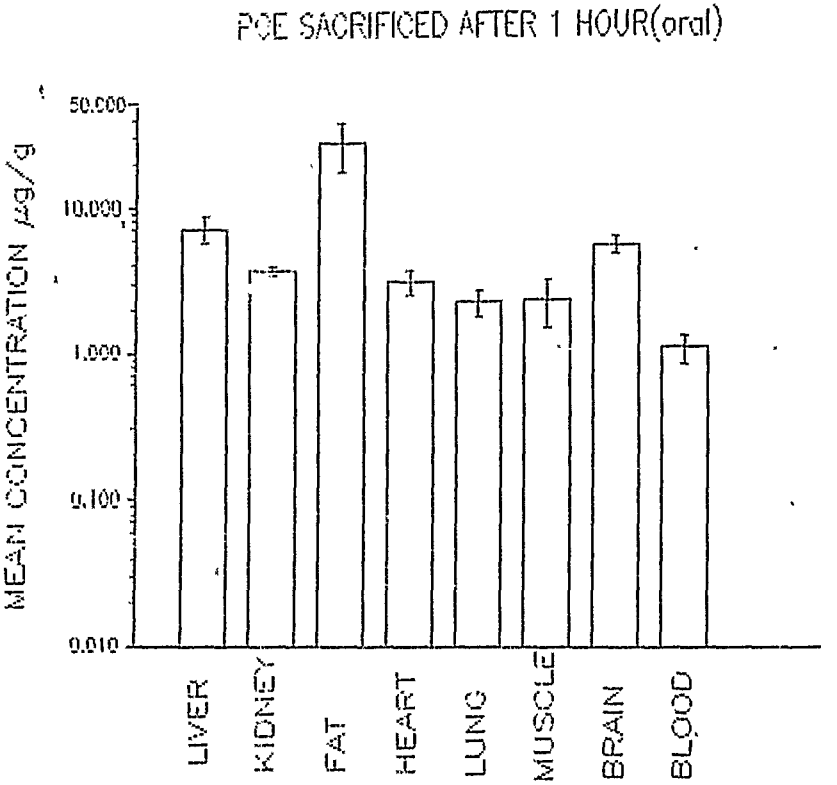

PCE SACRIFICED AFTER 1 HOUR(oral)
MEAN CONCENTRATION μg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-13

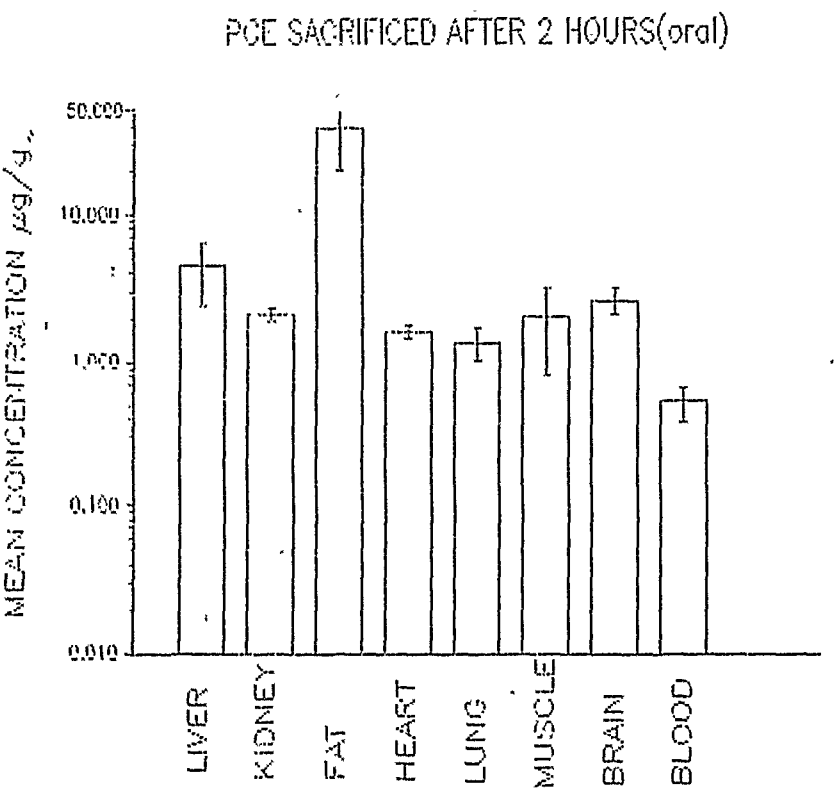

PCE SACRIFICED AFTER 2 HOURS(oral)
MEAN CONCENTRATION μg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-14

POE SACRIFICED AFTER 4 HOURS(oral)
MEAN CONCENTRATION µg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-15

PCE SACRIFICED AFTER 6 HOURS(oral)
MEAN CONCENTRATION µg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-16

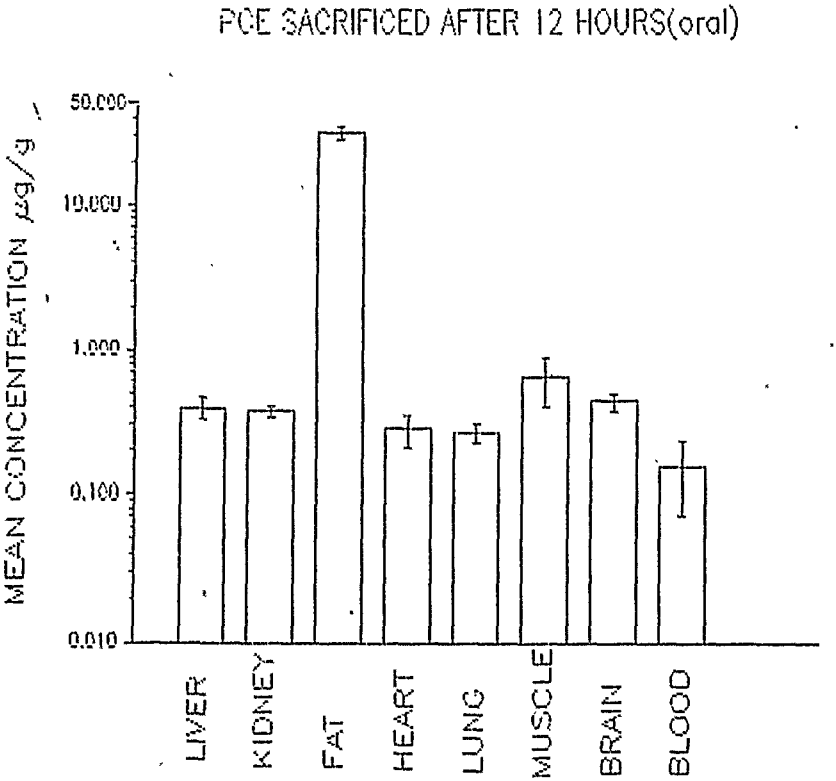

PCE SACRIFICED AFTER 12 HOURS(oral)
MEAN CONCENTRATION μg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-17

PCE SACRIFICED AFTER 18HOURS(oral)
MEAN CONCENTRATION μg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-18

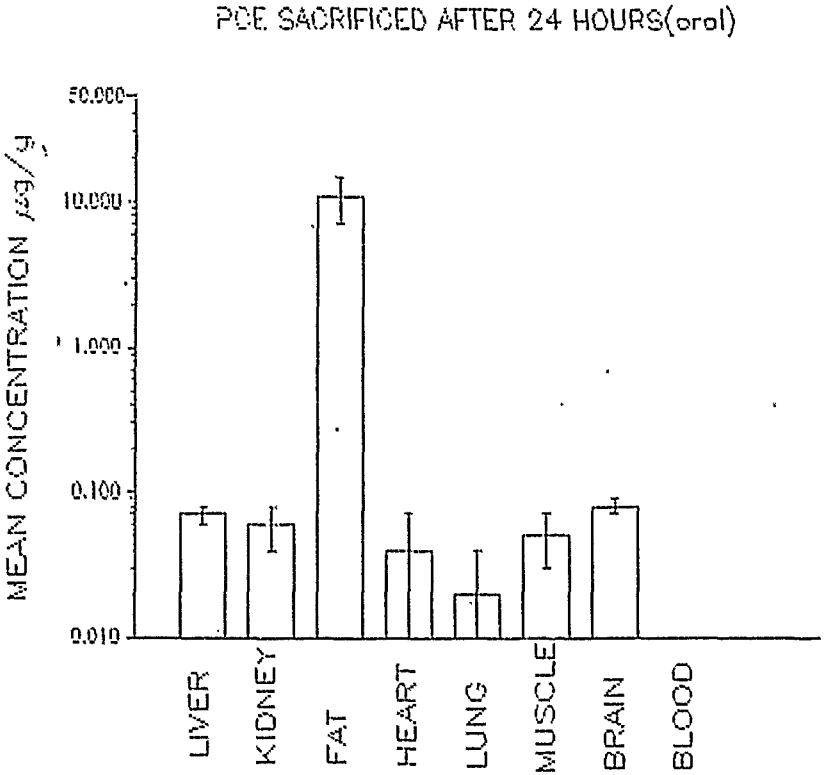

PCE SACRIFICED AFTER 24 HOURS(oral)
MEAN CONCENTRATION μg/g
50.000
10.000
1.000
0.100
0.010
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-19

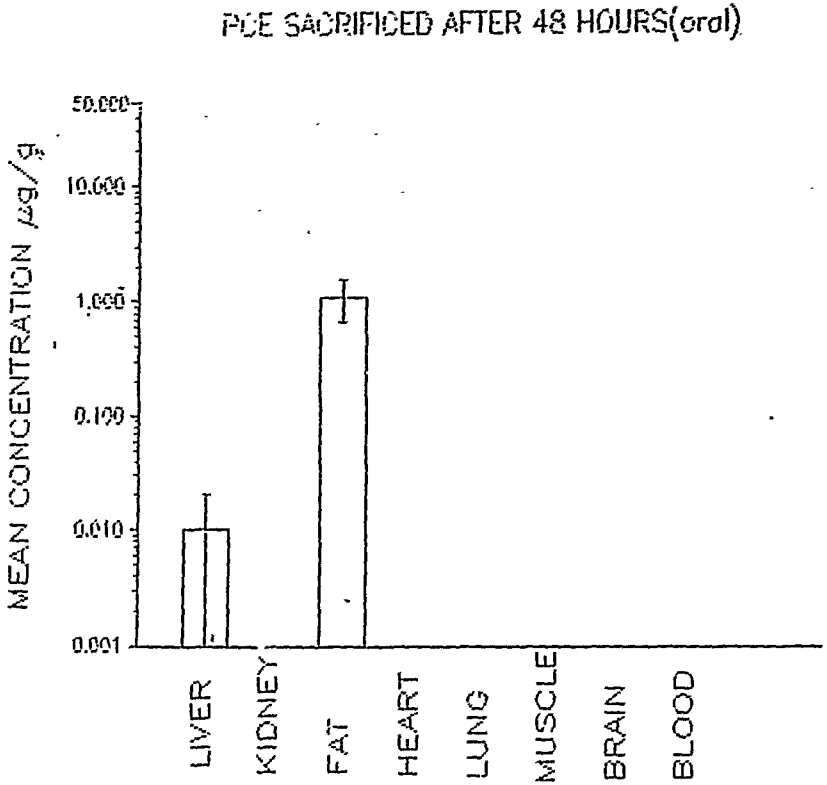

PCE SACRIFICED AFTER 48 HOURS(oral)
MEAN CONCENTRATION μg/g
50.000
10.000
1.000
0.100
0.010
0.001
LIVER
KIDNEY
FAT
HEART
LUNG
MUSCLE
BRAIN
BLOOD

Fig. G-20

PHARMACOKINETIC PARAMETERS
PER ORAL ADMINISTRATION
(10 mg/kg)

	AUC μ-min/g	T1/2 minutes	Cmax μg/g	Tmax minutes	Relative Bioavailability
LIVER	1969	266	13.8	10	0.83
KIDNEY	1287	333	6.2	10	0.59
FAT	50271	452	44.4	360	0.75
HEART	931	325	3.1	15	0.66
LUNG	732	326	2.3	60	0.62
MUSCLE	1120	303	2.4	60	0.68
BRAIN	1599	300	5.6	15	0.68
BLOOD	372	344	1.1	15	0.67

Table G-2

PHARMACOKINETIC PARAMETERS
PER IA ADMINISTRATION
(10 mg/kg)

	AUC μg-min/g	T1/2 minutes	Cmax μg/g	Tmax minutes
LIVER	2367	398	25.6	15
KIDNEY	2191	399	22.6	10
FAT	66962	463	67.0	60
HEART	1411	429	15.3	15
LUNG	1181	462	10.7	15
MUSCLE	1658	439	9.0	15
BRAIN	2354	407	25.2	15
BLOOD	555	472	5.5	15

Table G-3

TISSUE DISTRIBUTION OF PER IN LIVER, KIDNEY AND BRAIN
INTRAARTERIAL (10 MG/KG)

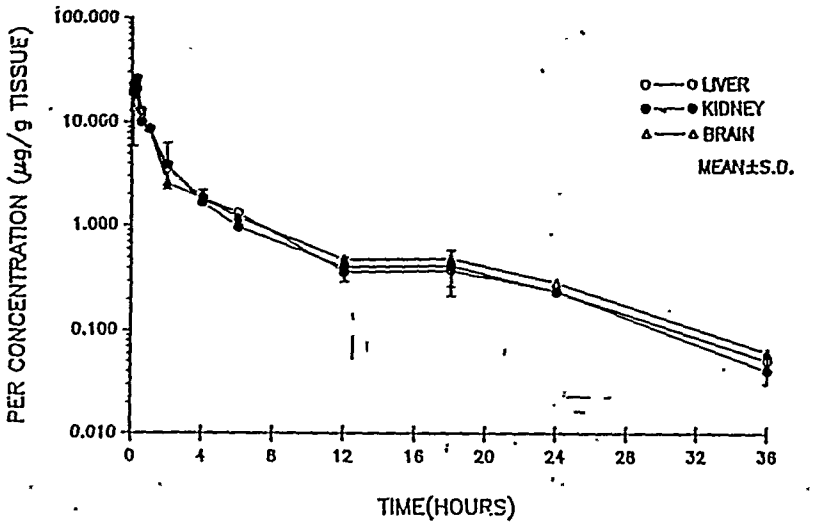


Fig. G-21

TISSUE DISTRIBUTION OF PER IN HEART, LUNGS AND MUSCLE
INTRAARTERIAL (10 MG/KG)

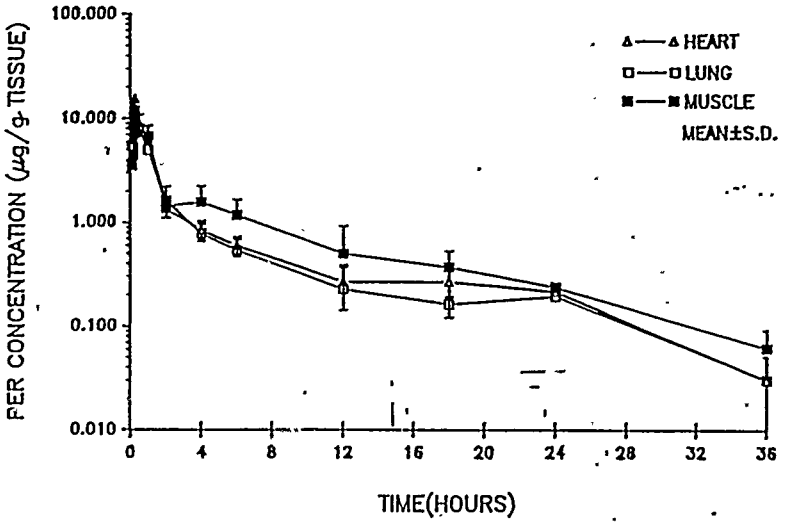


Fig. G-22

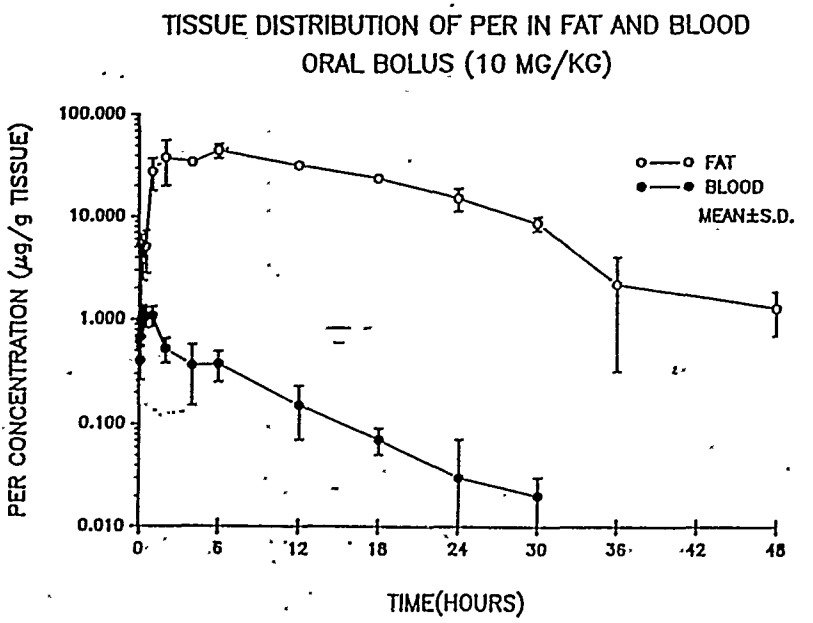

TISSUE DISTRIBUTION OF PER IN FAT AND BLOOD
ORAL BOLUS (10 MG/KG)
PER CONCENTRATION (µg/g TISSUE)
100.000
10.000
1.000
0.100
0.010
0
6
12
18
24
30
36
42
48
TIME(HOURS)
FAT
BLOOD
MEAN±S.D.

Fig. G-23

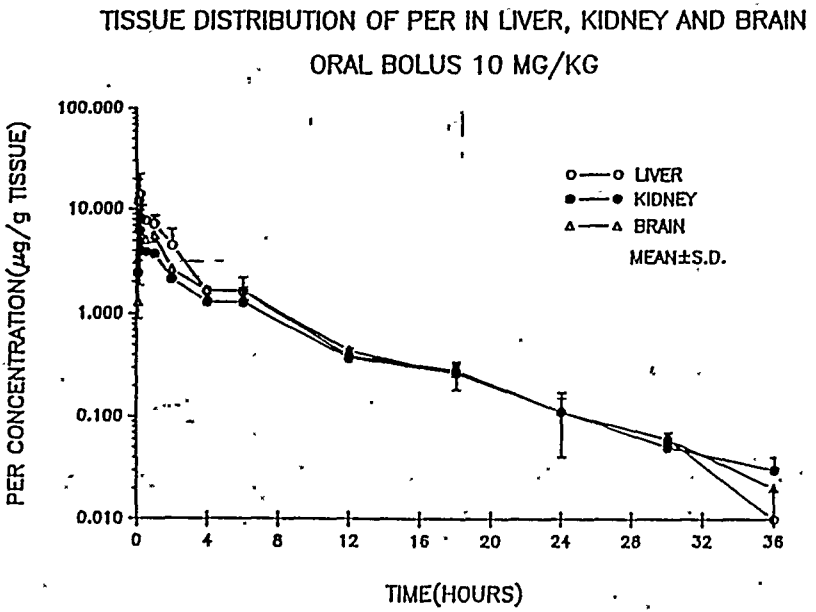

TISSUE DISTRIBUTION OF PER IN LIVER, KIDNEY AND BRAIN
ORAL BOLUS 10 MG/KG
PER CONCENTRATION(μg/g TISSUE)
100.000
10.000
1.000
0.100
0.010
0
4
8
12
16
20
24
28
32
36
TIME(HOURS)
LIVER
KIDNEY
BRAIN
MEAN±S.D.

Fig. G-24

TISSUE DISTRIBUTION OF PER-IN HEART, LUNG AND MUSCLE
ORAL BOLUS (10 MG/KG)

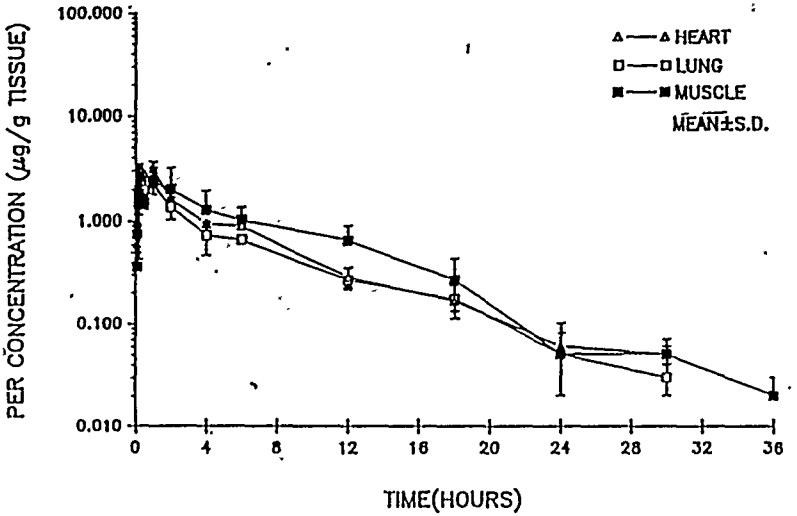


Fig. G-25

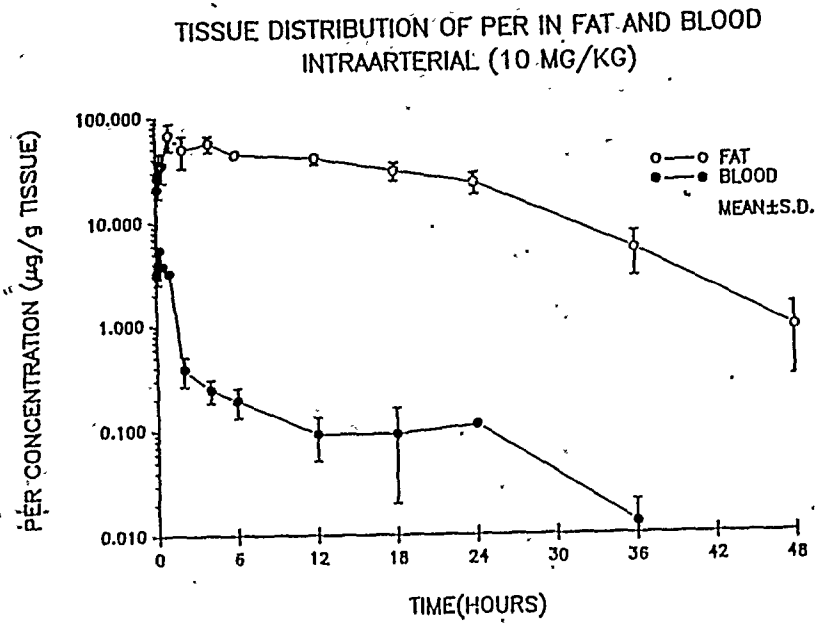

TISSUE DISTRIBUTION OF PER IN FAT AND BLOOD
INTRAARTERIAL (10 MG/KG)
PER CONCENTRATION (µg/g TISSUE)
100.000
10.000
1.000
0.100
0.010
0
6
12
18
24
30
36
42
48
TIME(HOURS)
FAT
BLOOD
MEAN±S.D.

Fig. G-26

APPENDIX H

STUDIES OF THE TISSUE UPTAKE, DISPOSITION,
AND ELIMINATION OF TET IN RATS

1) Following intraarterial administration

2) Following oral administration

PHARMACOKINETIC PARAMETERS OF INTRAARTERIAL ADMINISTRATION (10 MG/KG)

	AUC	T1/2		Cmax	Tmax	CL	Vd
	ug.min/g	min	hrs	ug/g	min	ml.min/kg	L/kg
LIVER	794.25	97.97	1.63	19.99	5.00	12.59	1.78
KIDNEY	829.77	93.69	1.56	26.07	5.00	12.05	1.63
FAT	10277.20	164.04	2.73	65.02	60.00	0.97	0.23
HEART	650.50	105.71	1.76	21.86	5.00	15.37	2.34
LUNG	400.87	89.63	1.49	8.46	5.00	24.95	3.23
MUSCLE	381.06	106.05	1.77	4.66	5.00	26.24	4.02
BRAIN	519.96	83.87	1.40	12.85	5.00	19.23	2.33
BLOOD	248.32	70.47	1.17	5.54	5.00	40.27	4.10

Table II-1

PHARMACOKINETIC PARAMETERS OF ORAL ADMINISTRATION (10 MG/KG)

	AUC	T1/2		Cmax	Tmax	CL	Vd
	ug.min/g	min	hrs	ug/g	min	ml.min/kg	L/kg
LIVER	289.95	61.83	1.03	4.83	15.00	34.49	3.08
KIDNEY	131.15	63.61	1.06	1.79	15.00	76.25	7.00
FAT	2108.26	215.97	3.60	6.07	30.00	4.74	1.48
HEART	111.629	65.758	1.10	1.83	15.00	89.58	8.50
LUNG	77.79	48.98	0.82	1.37	30.00	128.55	9.09
MUSCLE	75.156	91.14	1.52	0.81	15.00	133.06	17.50
BRAIN	116.347	71.49	1.19	1.65	15.00	85.95	8.87
BLOOD	65.54	58.567	0.98	1.02	15.00	152.58	12.89

Table H-2

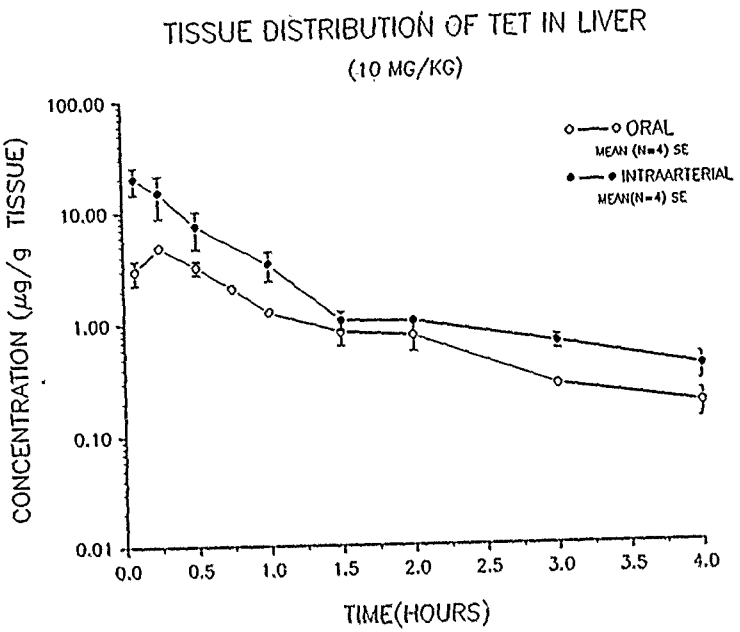

TISSUE DISTRIBUTION OF TET IN LIVER
(10 MG/KG)
100.00
10.00
1.00
0.10
0.01
CONCENTRATION (μg/g TISSUE)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
TIME(HOURS)
ORAL
MEAN (N=4) SE
INTRAARTERIAL
MEAN(N=4) SE

Fig. H-1

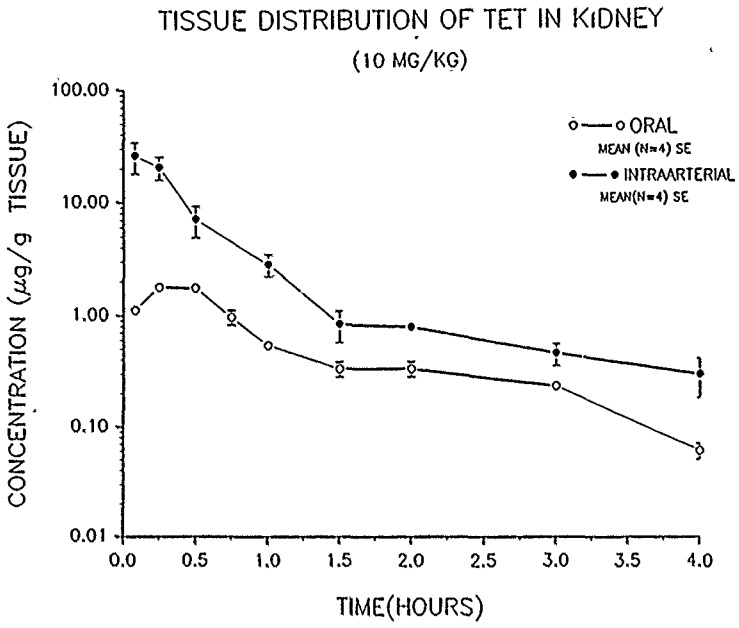

TISSUE DISTRIBUTION OF TET IN KIDNEY
(10 MG/KG)
CONCENTRATION (μg/g TISSUE)
100.00
10.00
1.00
0.10
0.01
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
TIME(HOURS)
o—o ORAL
MEAN (N=4) SE
•—• INTRAARTERIAL
MEAN(N=4) SE

Fig. H-2

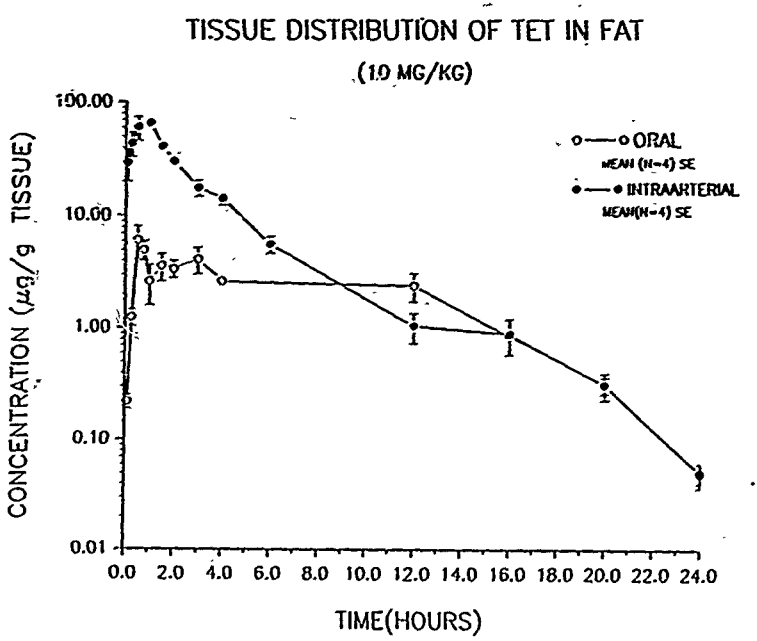

TISSUE DISTRIBUTION OF TET IN FAT
(10 MG/KG)
CONCENTRATION (µg/g TISSUE)
100.00
10.00
1.00
0.10
0.01
0.0 2.0 4.0 6.0 8.0 10.0 12.0 14.0 16.0 18.0 20.0 22.0 24.0
TIME(HOURS)
ORAL
MEAN (N=4) SE
INTRAARTERIAL
MEAN(N=4) SE

Fig. II-3

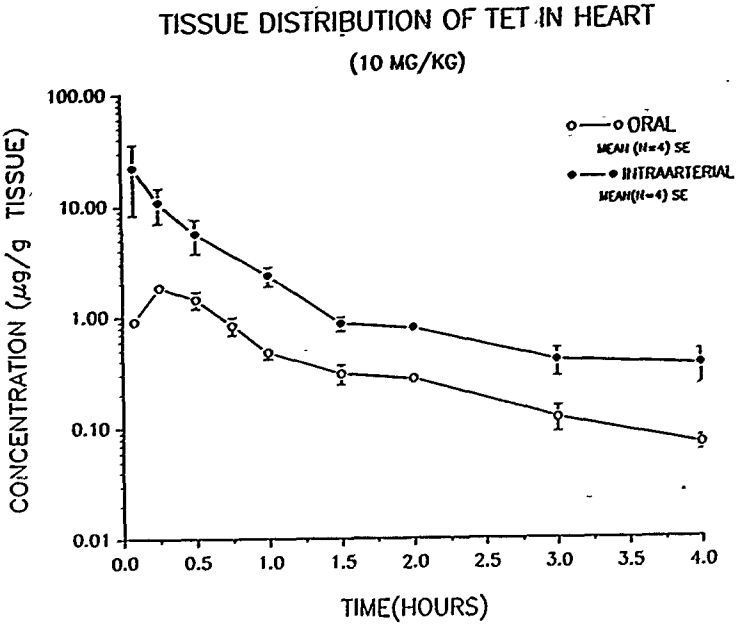

TISSUE DISTRIBUTION OF TET IN HEART
(10 MG/KG)
100.00
10.00
1.00
0.10
0.01
CONCENTRATION (μg/g TISSUE)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
TIME(HOURS)
ORAL
MEAN (N=4) SE
INTRAARTERIAL
MEAN(N=4) SE

Fig. II-4

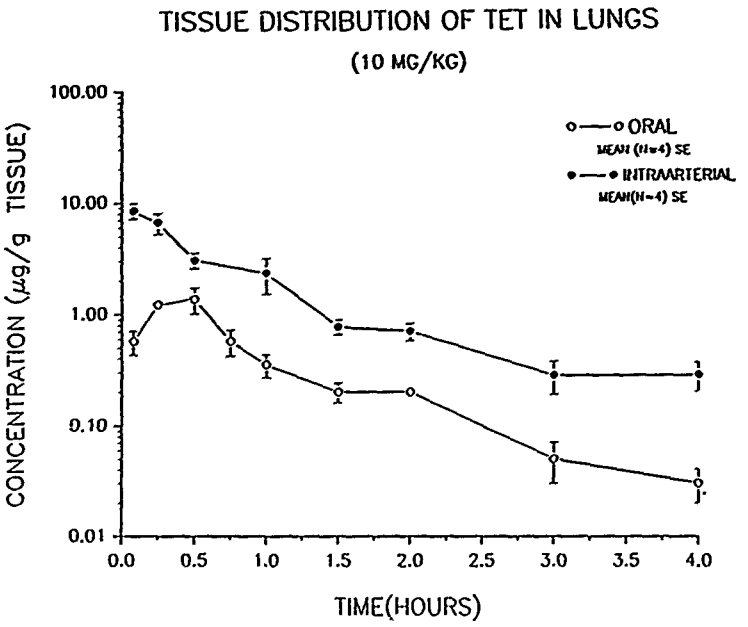

TISSUE DISTRIBUTION OF TET IN LUNGS
(10 MG/KG)
CONCENTRATION (μg/g TISSUE)
100.00
10.00
1.00
0.10
0.01
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
TIME(HOURS)
ORAL
MEAN (N=4) SE
INTRAARTERIAL
MEAN(N=4) SE

Fig. H-5

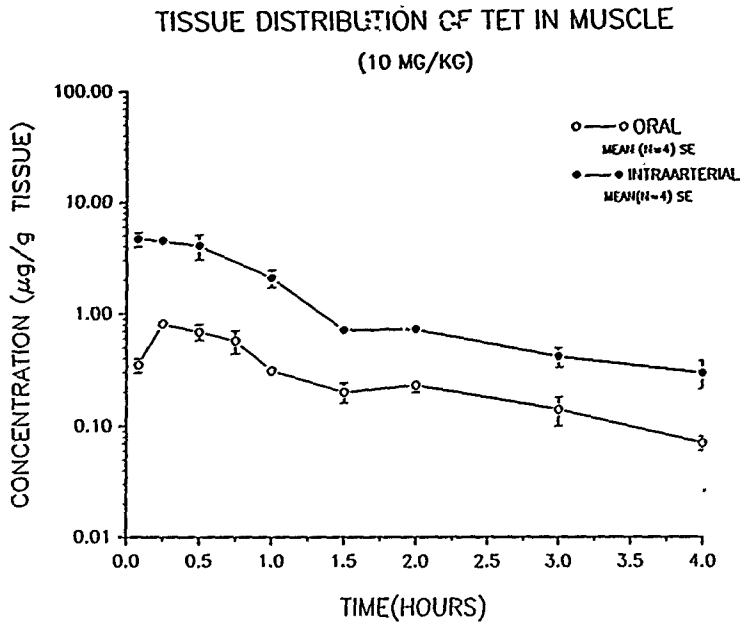

TISSUE DISTRIBUTION OF TET IN MUSCLE
(10 MG/KG)
CONCENTRATION (μg/g TISSUE)
100.00
10.00
1.00
0.10
0.01
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
TIME(HOURS)
ORAL
MEAN (N=4) SE
INTRAARTERIAL
MEAN(N=4) SE

Fig. II-6

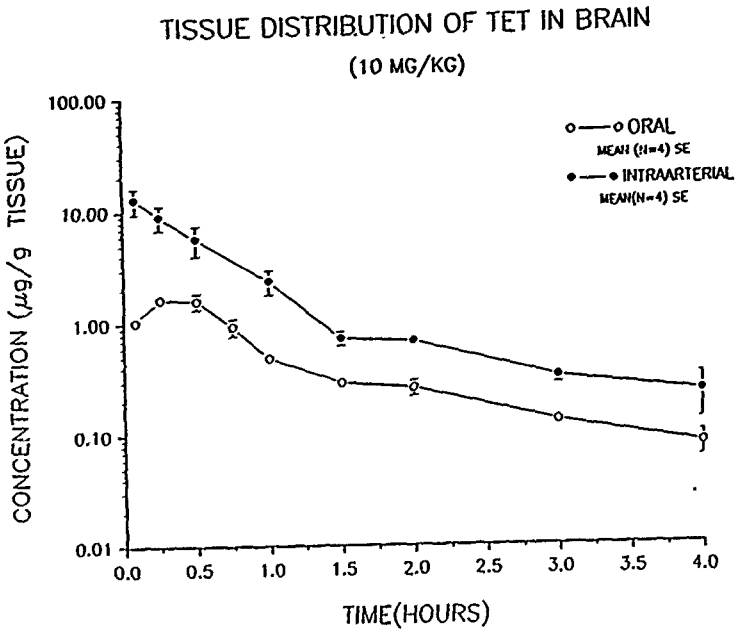

TISSUE DISTRIBUTION OF TET IN BRAIN
(10 MG/KG)
CONCENTRATION (μg/g TISSUE)
100.00
10.00
1.00
0.10
0.01
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
TIME(HOURS)
ORAL
MEAN (N=4) SE
INTRAARTERIAL
MEAN(N=4) SE

Fig. H-7

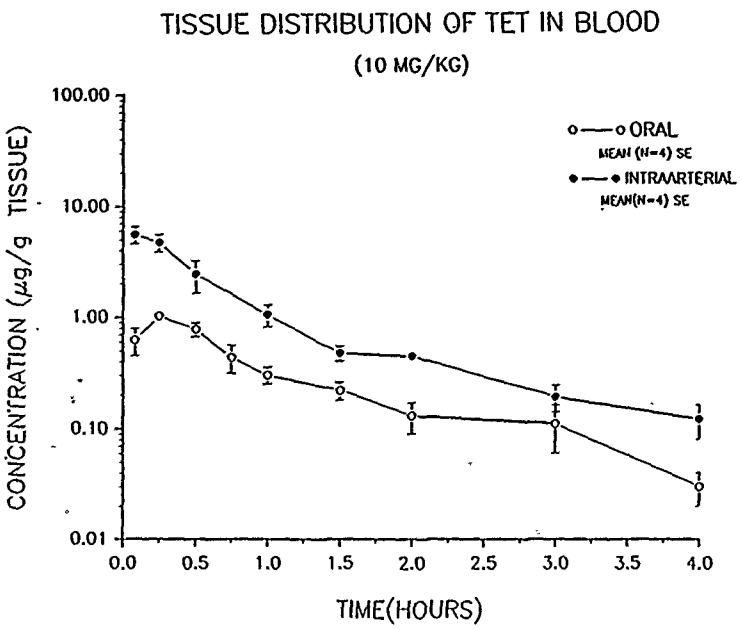

TISSUE DISTRIBUTION OF TET IN BLOOD
(10 MG/KG)
CONCENTRATION (μg/g TISSUE)
100.00
10.00
1.00
0.10
0.01
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
TIME(HOURS)
o—o ORAL
MEAN (N=4) SE
•—• INTRAARTERIAL
MEAN(N=4) SE

Fig. II-8

APPENDIX I

INTERSPECIES COMPARISONS OF THE PHARMACOKINETICS OF PCE

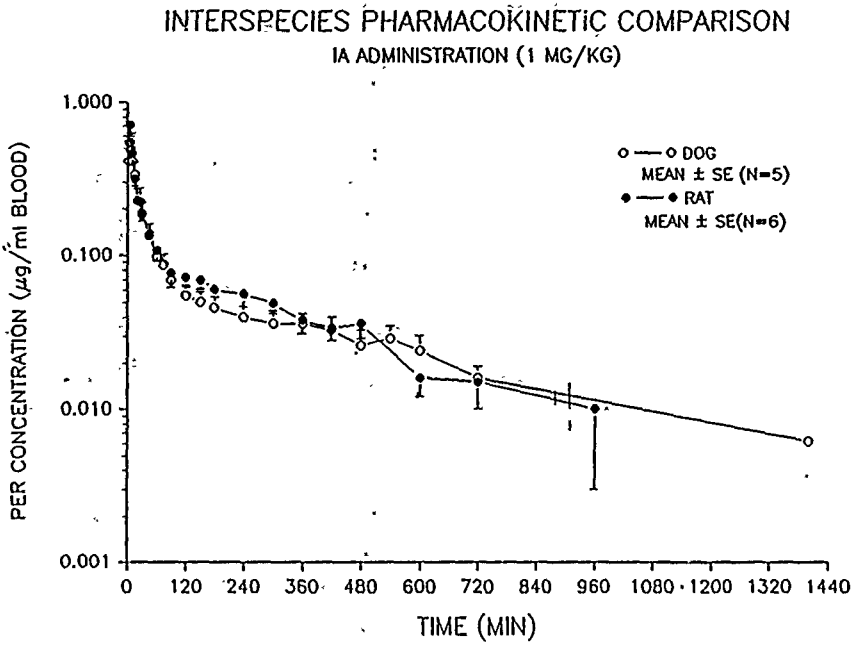

INTERSPECIES PHARMACOKINETIC COMPARISON
IA ADMINISTRATION (1 MG/KG)
PER CONCENTRATION (μg/ml BLOOD)
1.000
0.100
0.010
0.001
0 120 240 360 480 600 720 840 960 1080 1200 1320 1440
TIME (MIN)
o — o DOG
MEAN ± SE (N=5)
• — • RAT
MEAN ± SE(N=6)

Fig. I-1

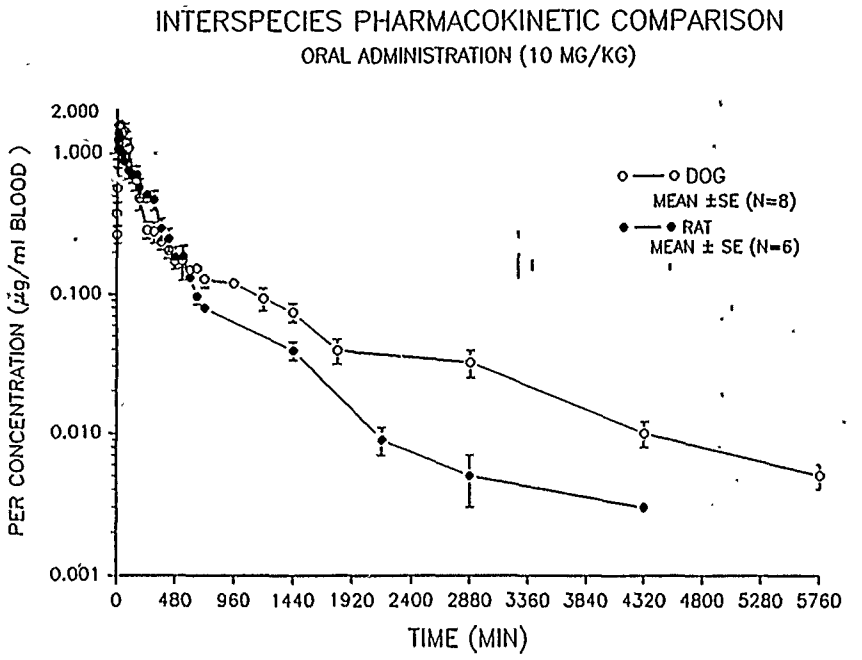

INTERSPECIES PHARMACOKINETIC COMPARISON
ORAL ADMINISTRATION (10 MG/KG)
PER CONCENTRATION (µg/ml BLOOD)
2.000
1.000
0.100
0.010
0.001
DOG
MEAN ±SE (N=8)
RAT
MEAN ± SE (N=6)
0
480
960
1440
1920
2400
2880
3360
3840
4320
4800
5280
5760
TIME (MIN)

Fig. I-2

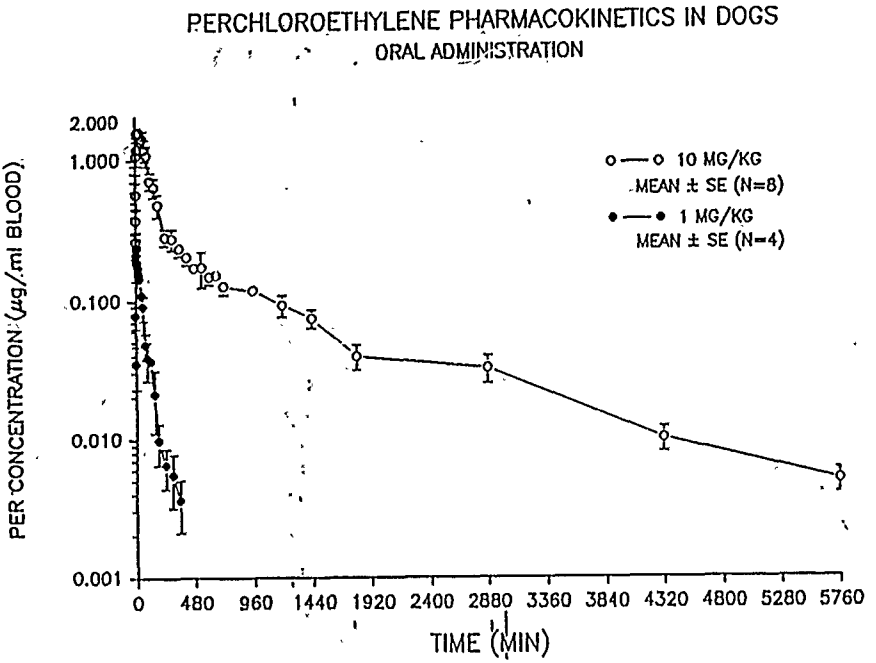

PERCHLOROETHYLENE PHARMACOKINETICS IN DOGS
ORAL ADMINISTRATION
PER CONCENTRATION (μg/ml BLOOD)
2.000
1.000
0.100
0.010
0.001
o—o 10 MG/KG
MEAN ± SE (N=8)
•—• 1 MG/KG
MEAN ± SE (N=4)
0
480
960
1440
1920
2400
2880
3360
3840
4320
4800
5280
5760
TIME (MIN)

Fig. I-3

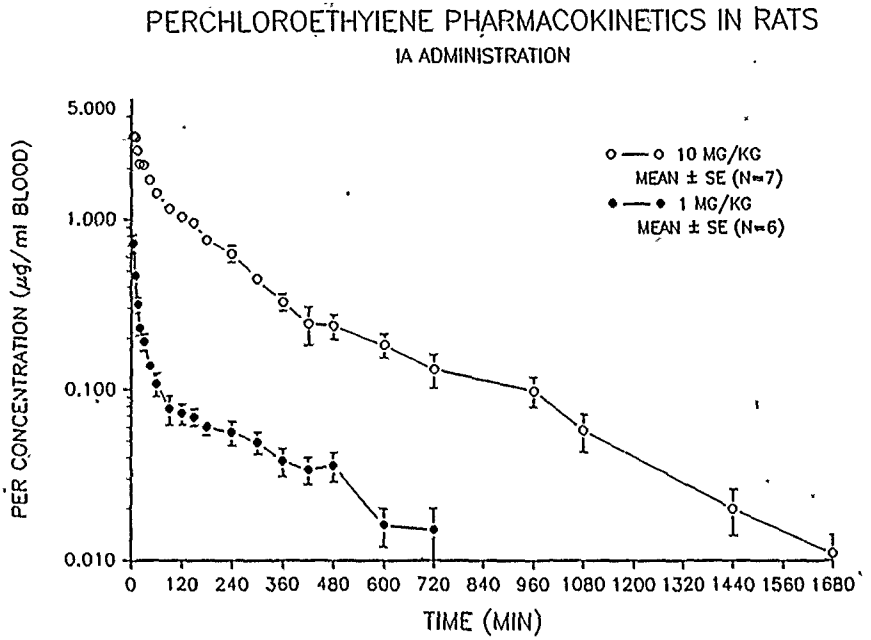

PERCHLOROETHYIENE PHARMACOKINETICS IN RATS
IA ADMINISTRATION
PER CONCENTRATION (µg/ml BLOOD)
5.000
1.000
0.100
0.010
0 120 240 360 480 600 720 840 960 1080 1200 1320 1440 1560 1680
TIME (MIN)
o — o 10 MG/KG
MEAN ± SE (N=7)
• — • 1 MG/KG
MEAN ± SE (N=6)

Fig. I-4

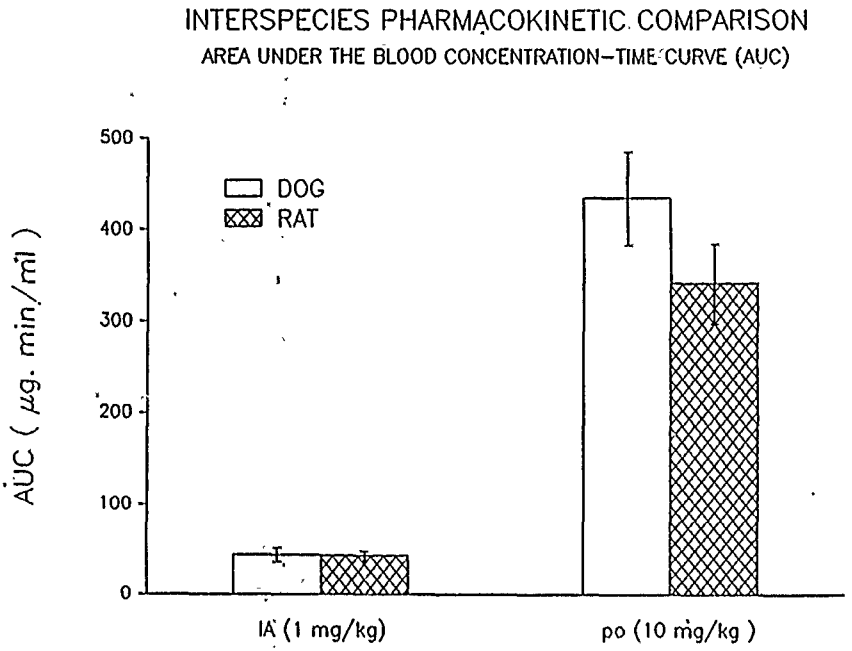

INTERSPECIES PHARMACOKINETIC COMPARISON
AREA UNDER THE BLOOD CONCENTRATION–TIME CURVE (AUC)
AUC (μg. min/ml)
500
400
300
200
100
0
DOG
RAT
IA (1 mg/kg)
po (10 mg/kg)

Fig. I-5

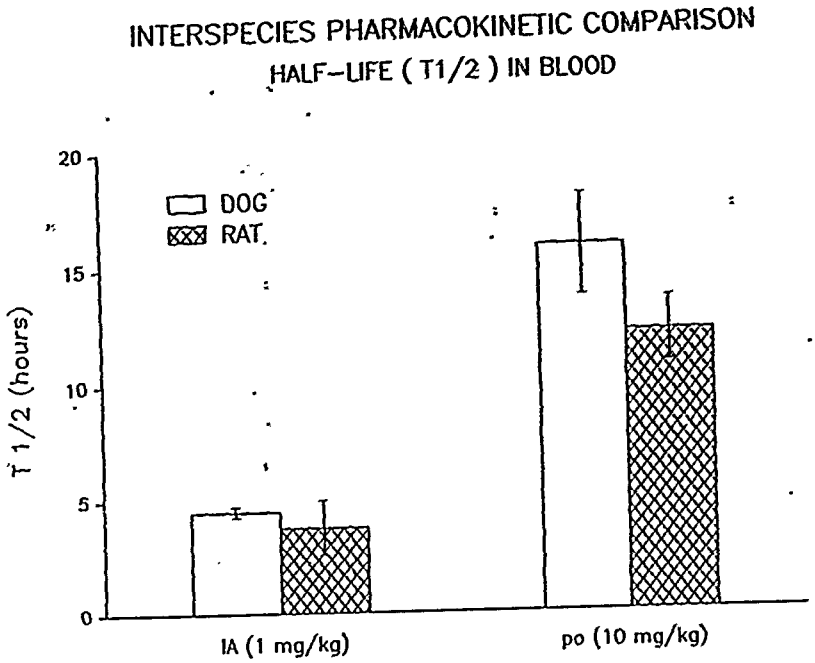

INTERSPECIES PHARMACOKINETIC COMPARISON
HALF-LIFE (T1/2) IN BLOOD
DOG
RAT
T 1/2 (hours)
20
15
10
5
0
IA (1 mg/kg)
po (10 mg/kg)

Fig. I-6

INTERSPECIES PHARMACOKINETIC COMPARISON

MAXIMUM BLOOD CONCENTRATION (Cmax)

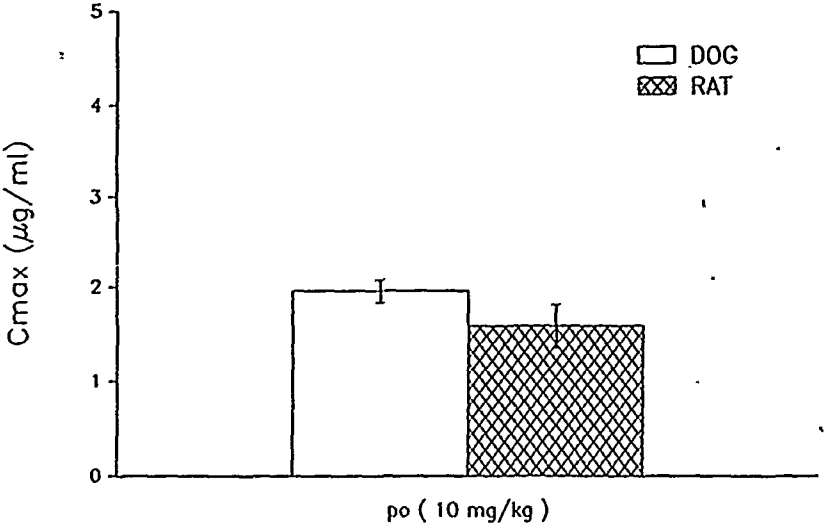


Fig. I-7

PER 3 MG/KG PHARMACOKINETIC PARAMETERS PO & IA ADMINISTRATION FOR RATS & DOGS

		AUC	T1/2		Cmax	Tmax	CL	Vd
		ug.min/ml	min.	hrs.	ug/ml	min.	ml.min/kg	L/kg
RATS	PO	185.973	971.563	16.193	0.462	25.000	16.348	23.205
DOGS	PO	228.038	1437.245	23.954	1.208	15	13.785	28.2
RATS	IA	154.108	642.092	10.702	4.405	3.23	20.256	18.276
DOGS	IA	206.999	1472.203	24.537	4.028	2.75	14.672	31.82

Table I-1

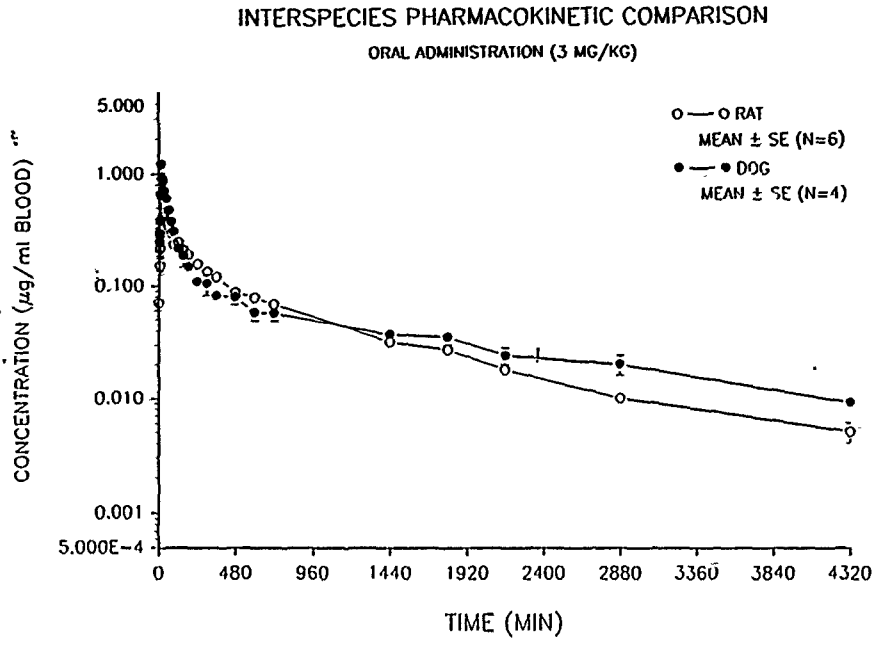

INTERSPECIES PHARMACOKINETIC COMPARISON
ORAL ADMINISTRATION (3 MG/KG)
O — O RAT
MEAN ± SE (N=6)
● — ● DOG
MEAN ± SE (N=4)
CONCENTRATION (μg/ml BLOOD)
5.000
1.000
0.100
0.010
0.001
5.000E-4
0
480
960
1440
1920
2400
2880
3360
3840
4320
TIME (MIN)

Fig. I-8

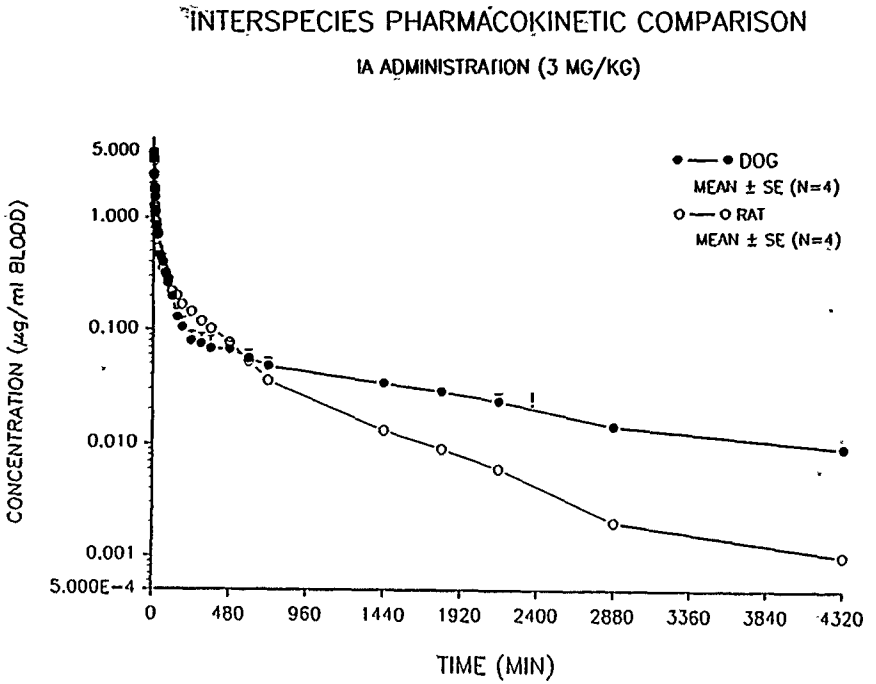

INTERSPECIES PHARMACOKINETIC COMPARISON
IA ADMINISTRATION (3 MG/KG)
CONCENTRATION (μg/ml BLOOD)
5.000
1.000
0.100
0.010
0.001
5.000E-4
0
480
960
1440
1920
2400
2880
3360
3840
4320
TIME (MIN)
DOG
MEAN ± SE (N=4)
RAT
MEAN ± SE (N=4)

Fig. I-9

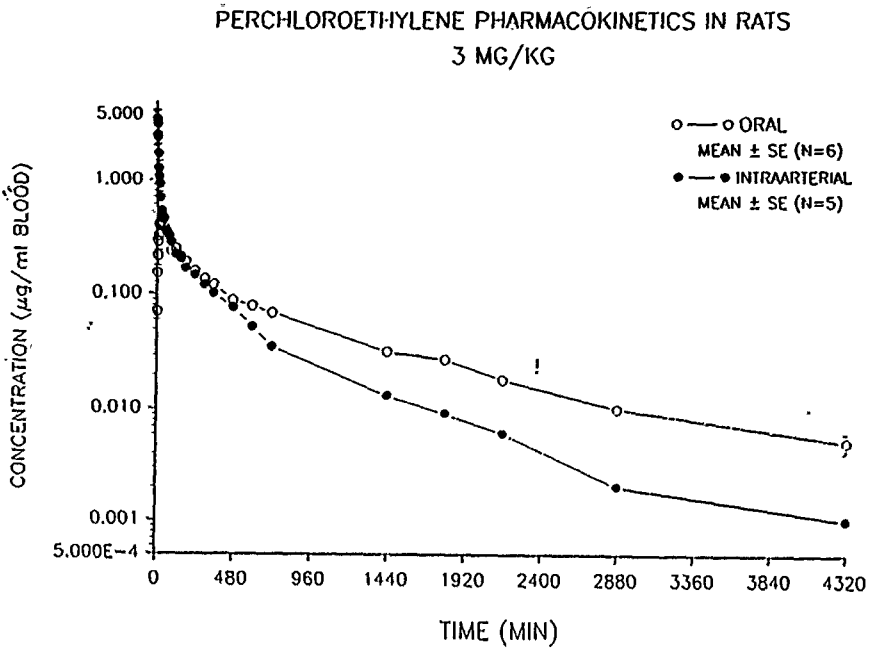

PERCHLOROETHYLENE PHARMACOKINETICS IN RATS
3 MG/KG
o — o ORAL
MEAN ± SE (N=6)
• — • INTRAARTERIAL
MEAN ± SE (N=5)
CONCENTRATION (μg/ml BLOOD)
5.000
1.000
0.100
0.010
0.001
5.000E-4
0
480
960
1440
1920
2400
2880
3360
3840
4320
TIME (MIN)

Fig. I-10

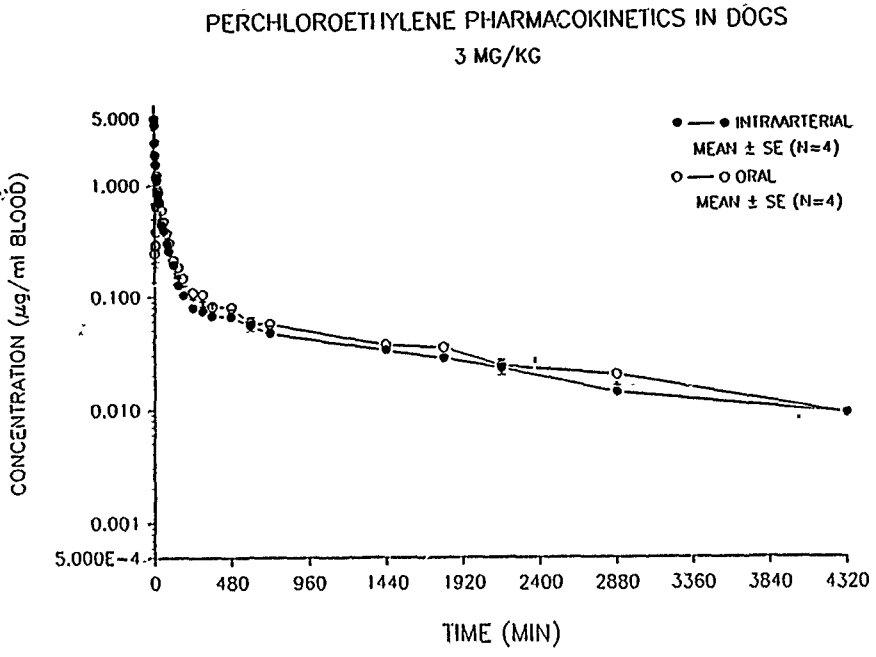

PERCHLOROETHYLENE PHARMACOKINETICS IN DOGS
3 MG/KG
● — ● INTRAARTERIAL
MEAN ± SE (N=4)
o — o ORAL
MEAN ± SE (N=4)
CONCENTRATION (μg/ml BLOOD)
5.000
1.000
0.100
0.010
0.001
5.000E-4
0
480
960
1440
1920
2400
2880
3360
3840
4320
TIME (MIN)

Fig. I-11

APPENDIX J

INTERSPECIES COMPARISONS OF THE PHARMACOKINETICS OF TET

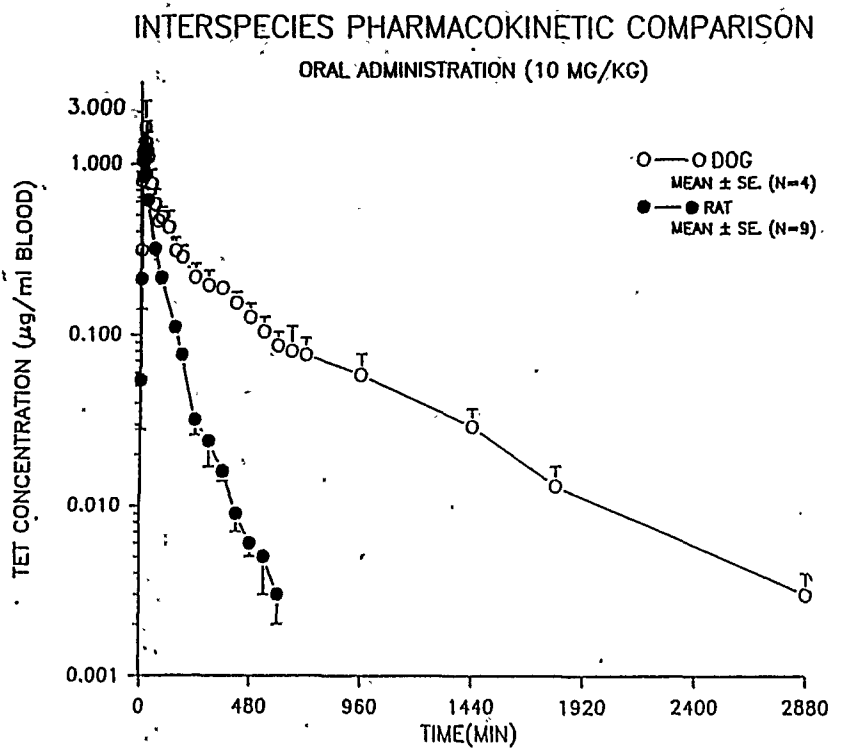

INTERSPECIES PHARMACOKINETIC COMPARISON
ORAL ADMINISTRATION (10 MG/KG)
TET CONCENTRATION (μg/ml BLOOD)
3.000
1.000
0.100
0.010
0.001
0
480
960
1440
1920
2400
2880
TIME(MIN)
O—O DOG
MEAN ± SE. (N=4)
●—● RAT
MEAN ± SE. (N=9)

Fig. J-1

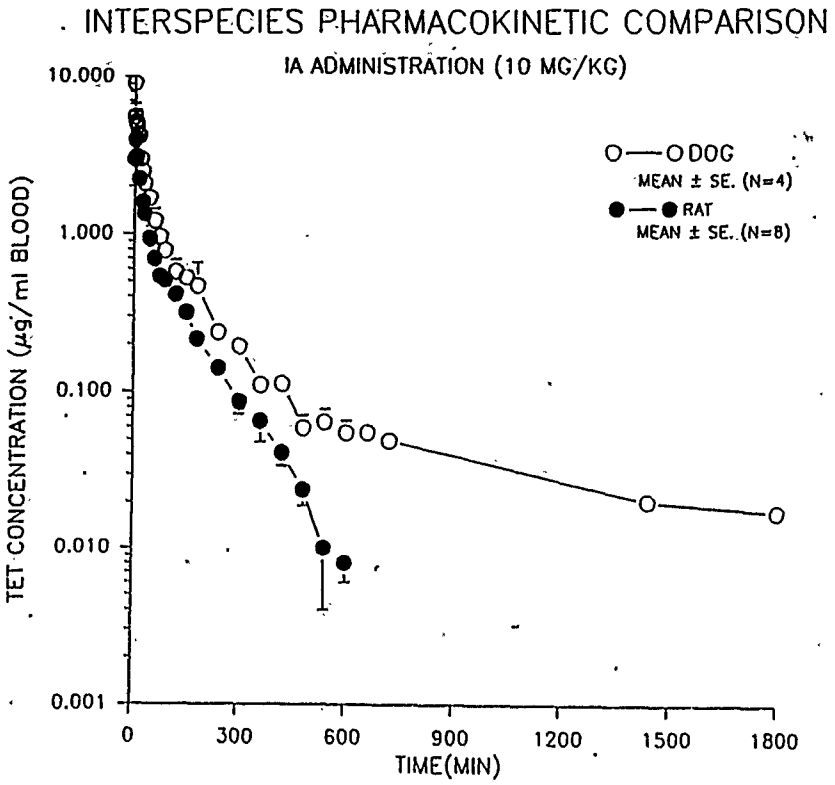

INTERSPECIES PHARMACOKINETIC COMPARISON
IA ADMINISTRATION (10 MG/KG)
O—O DOG
MEAN ± SE. (N=4)
●—● RAT
MEAN ± SE. (N=8)
TET CONCENTRATION (μg/ml BLOOD)
10.000
1.000
0.100
0.010
0.001
0
300
600
900
1200
1500
1800
TIME(MIN)

Fig. J-2

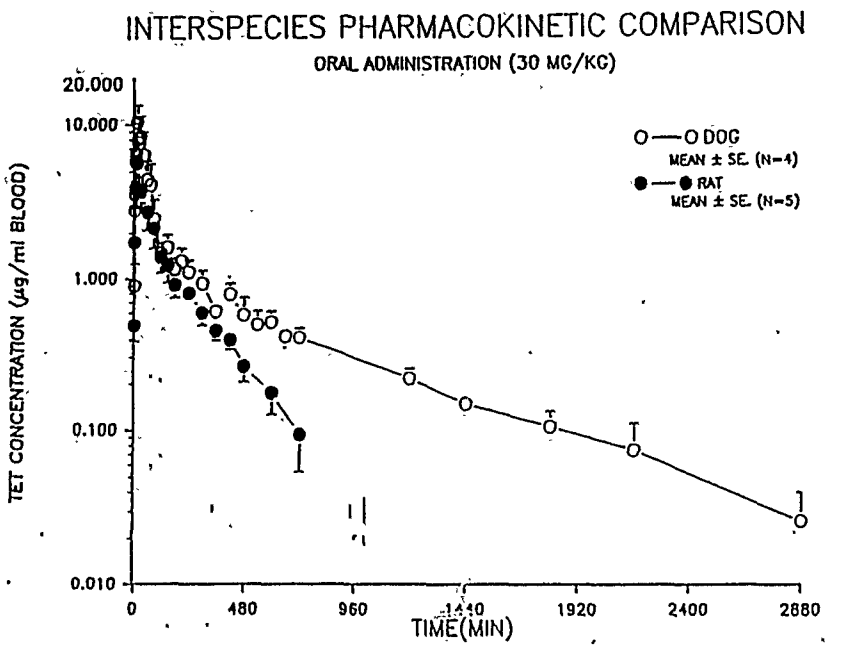

INTERSPECIES PHARMACOKINETIC COMPARISON
ORAL ADMINISTRATION (30 MG/KG)
TET CONCENTRATION (µg/ml BLOOD)
20.000
10.000
1.000
0.100
0.010
0
480
960
1440
1920
2400
2880
TIME(MIN)
O—O DOG
MEAN ± SE. (N=4)
●—● RAT
MEAN ± SE. (N=5)

Fig. J-3

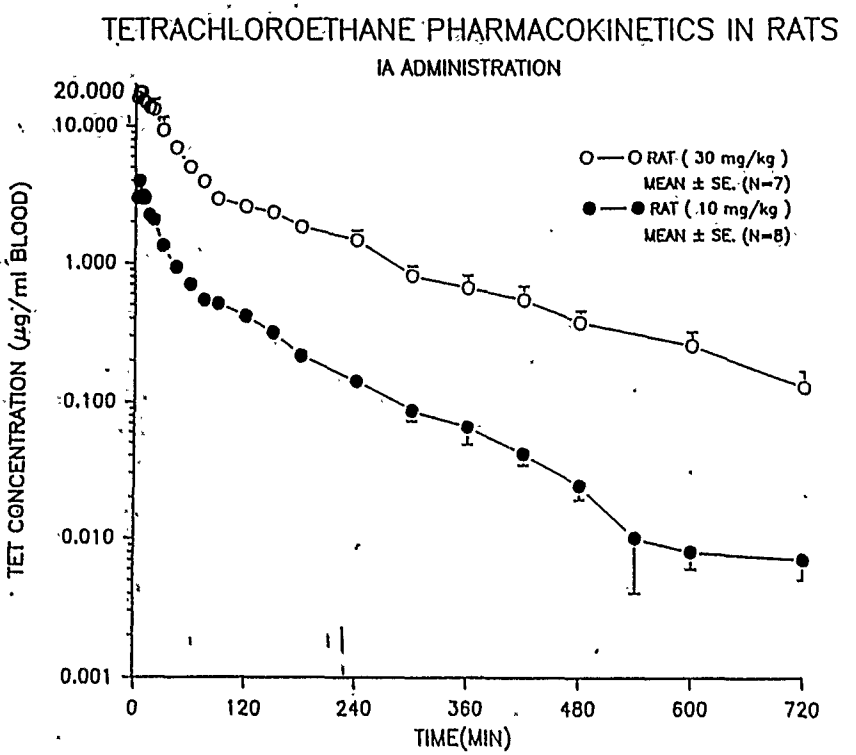

TETRACHLOROETHANE PHARMACOKINETICS IN RATS
IA ADMINISTRATION
O—O RAT (30 mg/kg)
MEAN ± SE. (N=7)
●—● RAT (10 mg/kg)
MEAN ± SE. (N=8)
TET CONCENTRATION (μg/ml BLOOD)
20.000
10.000
1.000
0.100
0.010
0.001
0
120
240
360
480
600
720
TIME(MIN)

Fig. J-4

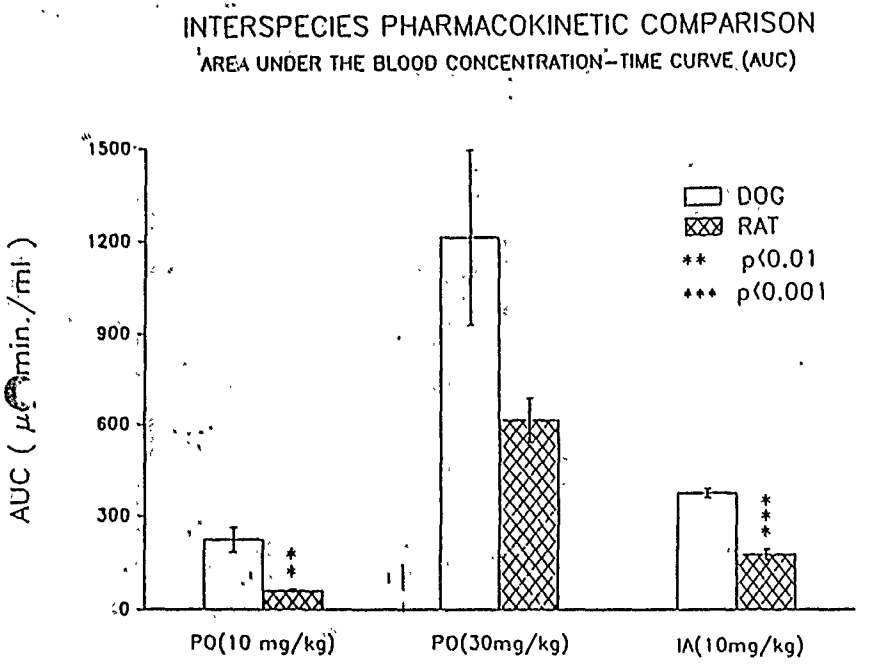

INTERSPECIES PHARMACOKINETIC COMPARISON
AREA UNDER THE BLOOD CONCENTRATION-TIME CURVE (AUC)
AUC (μg·min./ml)
1500
1200
900
600
300
0
DOG
RAT
** p<0.01
*** p<0.001
PO(10 mg/kg)
PO(30mg/kg)
IA(10mg/kg)

Fig. J-5

INTERSPECIES PHARMACOKINETIC COMPARISON

TET HALF-LIFE (T1/2) IN BLOOD

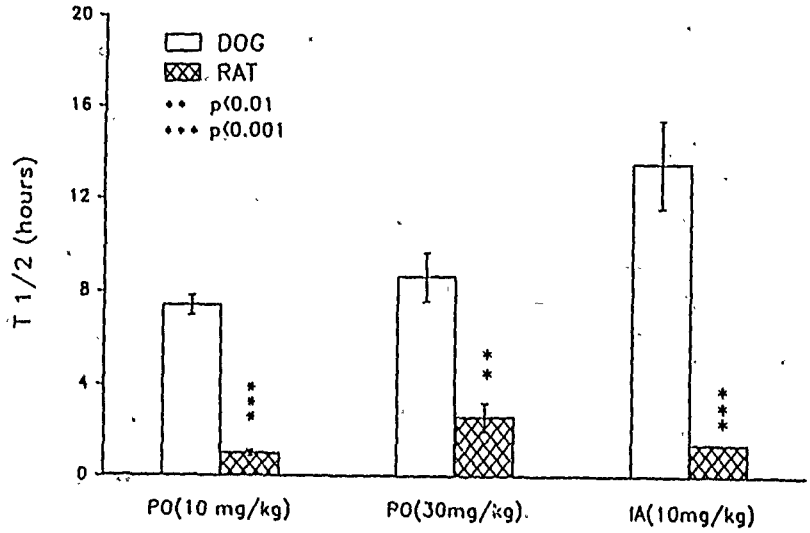


Fig. J-6

INTERSPECIES PHARMACOKINETIC COMPARISON
MAXIMUM BLOOD CONCENTRATION (Cmax)

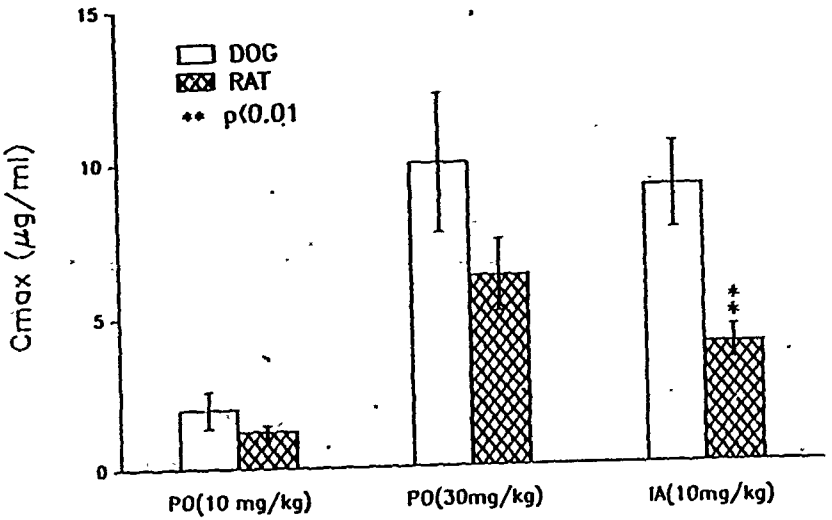


Fig. I-7

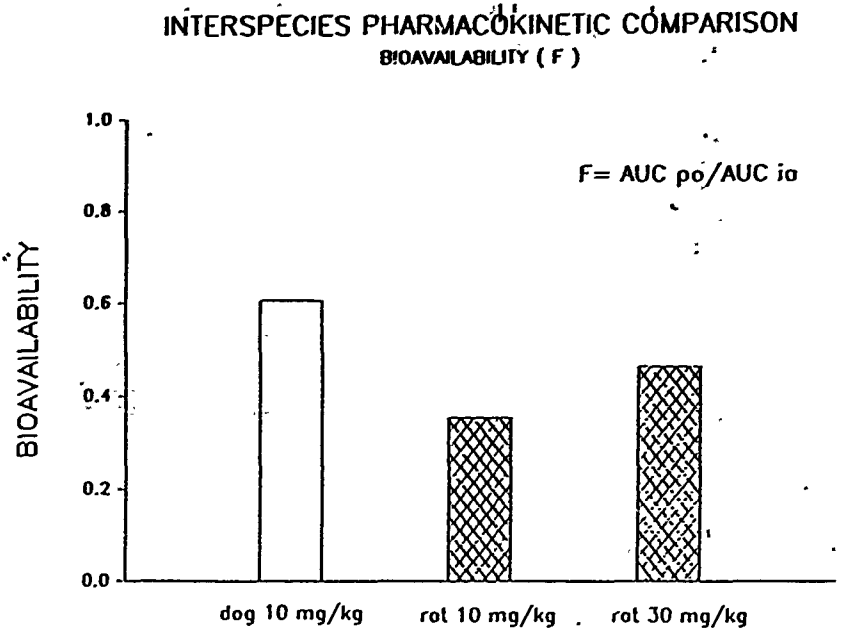

INTERSPECIES PHARMACOKINETIC COMPARISON
BIOAVAILABILITY (F)
F= AUC po/AUC iv
BIOAVAILABILITY
1.0
0.8
0.6
0.4
0.2
0.0
dog 10 mg/kg
rat 10 mg/kg
rat 30 mg/kg

Fig. J-8

APPENDIX K

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL SIMULATIONS OF THE UPTAKE, DISPOSITION, AND ELIMINATION OF INHALED AND INGESTED HALOCARBONS

(also see Appendix A, B, and E)

Differential equation system

Non-eliminating organs:

$$V_i \frac{dC_i}{dt} = Q_i \left(C_T - \frac{C_i}{R_i}\right)$$

where i= H; BR; K; M; R; F

arterial blood

$$V_{BL} \frac{dC_{BL}}{dt} = Q_T \left(\frac{C_L}{R_L} - C_{BL}\right)$$

lung

$$V_L \frac{dC_L}{dt} = Q_T \left(C_V - \frac{C_L}{R_L}\right) + h \left(C_A - \frac{C_L}{R_A}\right)$$

liver

$$V_{Li} \frac{dC_{Li}}{dt} = Q_{Li} \left(C_{BL} - \frac{C_{Li}}{R_{Li}}\right) - \frac{V_m \frac{C_{Li}}{R_{Li}}}{k_m + \frac{C_{Li}}{R_{Li}}}$$

alveloar space

i./ intraarterial administration

$$V_A \frac{dC_A}{dt} = h \left(\frac{C_L}{R_A} - C_A\right) - VR_A C_A$$

ii./ inhalation

$$V_A \frac{dC_A}{dt} = VR_A C_{INH}\, \mu(t) - VR_A C_A + h \left(\frac{C_L}{R_A} - C_A\right)$$

if t<=120 min u(t)=1 else u(t)=0

venous blood

$$V_{BL} \frac{dC_V}{dt} = \sum Q_i \frac{C_i}{R_i} - Q_T \frac{C_L}{R_L}$$

where i=H; BR; Li; K; M; F; R

Calculation of partition coefficients from intraarterial data

R_R and R_A are taken from [1]

The part. coeff. of noneliminating compartments (except the lung) are calculated according to [2]:

$$R_i = \frac{AUC_i}{AUC_{BL}}$$

where i=H; BR; K; R; F

R_L is calculated integrating the differential equation of arterial blood:

$$\int_0^\infty V_{BL}\frac{dC_{BL}}{dt}dt = Q_T\left(\frac{\int_0^\infty C_L dt}{R_L} - \int_0^\infty C_{BL}dt\right)$$

$$V_{BL}C_{BL}^0 = Dose = Q_T\left(\frac{AUC_L}{R_L} - AUC_{BL}\right)$$

$$R_L = \frac{AUC_L}{\frac{Dose}{Q_T} + AUC_{BL}}$$

References

[1] Dallas,C.E. et al.: The Uptake and Elimination of 1,1,1-Trichloroethane during and following Inhalation Exposures in Rats. Toxicol.Appl.Pharmacol. 98, 385-397 (1989).

[2] Gallo,J.M. et al.: Area Method for the estimation of Partition Coefficient for Physiological Pharmacokinetic Parameters. J.Pharmacokin.Biopharm. 15,271-280 (1987).

R_{Li} is calculated from the differential equation of the liver

Definitions:

$$AUC_{LiBL}=\int_0^{\infty} C_{Li}C_{BL}dt; \quad AUC_{Li^2}=\int_0^{\infty} C_{Li}^2 dt; \quad \int CdC=\frac{C^2}{2}$$

the differential equation of the liver:

$$V_{Li}\frac{dC_{Li}}{dt}=Q_{Li}(C_{BL}-\frac{C_{Li}}{R_{Li}})-\frac{V_m C_{Li}}{k_m R_{Li}+C_{Li}}$$

rearranging gives:

$$V_{Li}k_m R_{Li}\frac{dC_{Li}}{dt}+V_{Li}C_{Li}\frac{dC_{Li}}{dt}=$$

$$=Q_{Li}k_m R_{Li}C_{BL}+Q_{Li}C_{Li}C_{BL}-Q_{Li}k_m R_{Li}\frac{C_{Li}}{R_{Li}}-Q_{Li}\frac{C_{Li}^2}{R_{Li}}-V_m C_{Li}$$

integrating from 0 to infinity:

$$0=V_{Li}k_m R_{Li}[C_{Li}]_0^{\infty}+V_{Li}[\frac{C_{Li}^2}{2}]_0^{\infty}=$$

$$=Q_{Li}k_m R_{Li}AUC_{BL}+Q_{Li}AUC_{LiBL}-$$

$$-Q_{Li}k_m AUC_{Li}-Q_{Li}\frac{AUC_{Li^2}}{R_{Li}}-V_m AUC_{Li}$$

after rearranging:

$$0=R_{Li}^2 Q_{Li}k_m AUC_{BL}+$$

$$+R_{Li}(Q_{Li}AUC_{LiBL}-Q_{Li}k_m AUC_{Li}-V_m AUC_{Li}-$$

$$-Q_{Li}AUC_{Li^2}$$

solving the equation:

$$a=Q_{Li}k_m AUC_{BL}$$

$$b=Q_{Li}AUC_{LiBL}-Q_{Li}k_m AUC_{Li}-V_m AUC_{Li}$$

$$c=-Q_{Li}AUC_{Li^2}$$

$$R_{Li}=\frac{-b\pm\sqrt{b^2-4ac}}{2a}$$

PHARMACOKINETIC PARAMETERS

PER INHALATION ADMINISTRATION

(2500 ppm =799 mg/kg)

	AUC	T1/2		Cmax	Tmax
	ug.min/g	min.	hrs.	ug/g	min.
LIVER	82945	461	7.68	292.72	1
KIDNEY	75314	455	7.58	185.18	15
FAT	397943	738.8	12.31	3696.36	120
HEART	72739	475	7.92	187.99	15
LUNG	57251	465	7.75	147.73	15
MUSCLE	61027	455	7.58	108.86	60
BRAIN	83089	451	7.52	224.12	15
BLOOD	29834	441	7.35	58.89	15

Table K-1

PHARMACOKINETIC PARAMETERS

PER INHALATION ADMINISTRATION

(500 ppm =165 mg/kg)

	AUC ug.min/g	T1/2 min.	hrs.	Cmax ug/g	Tmax min.
LIVER	31247	423	7.05	152.40	15
KIDNEY	25868	425	7.08	107.52	15
FAT	1493190	578	9.63	1536.30	240
HEART	29179	328	5.47	106.60	15
LUNG	18596	406	6.77	94.55	15
MUSCLE	24458	335	5.58	87.31	15
BRAIN	32975	455	7.58	173.89	15
BLOOD	8464	322	5.37	44.89	15

Table K-2

TISSUE DISTRIBUTION OF PER IN LIVER

AFTER 2 HOURS EXPOSURE

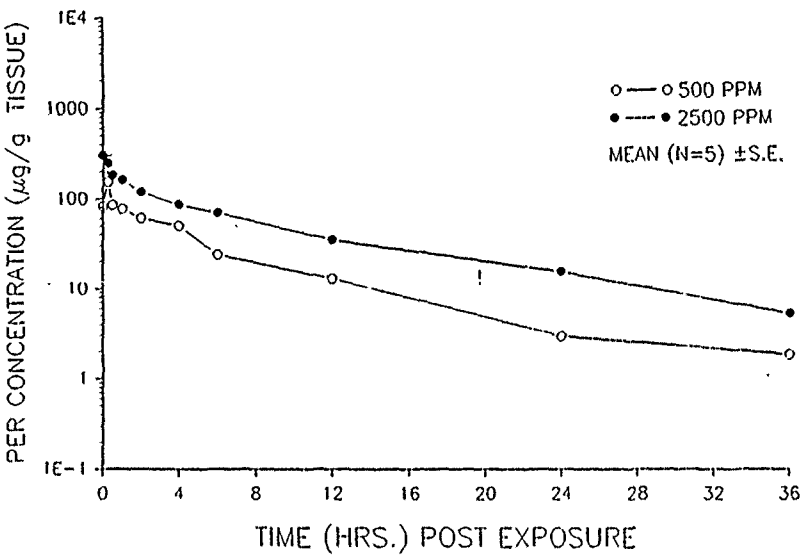


Fig. K-1

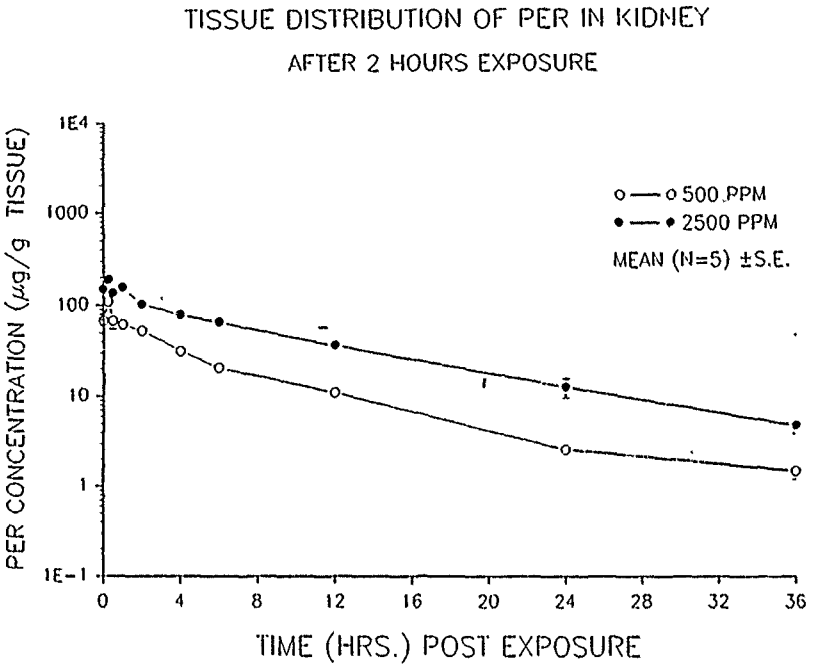

TISSUE DISTRIBUTION OF PER IN KIDNEY
AFTER 2 HOURS EXPOSURE
PER CONCENTRATION (μg/g TISSUE)
1E4
1000
100
10
1
1E-1
0 — o 500 PPM
• — • 2500 PPM
MEAN (N=5) ±S.E.
0
4
8
12
16
20
24
28
32
36
TIME (HRS.) POST EXPOSURE

Fig. K-2

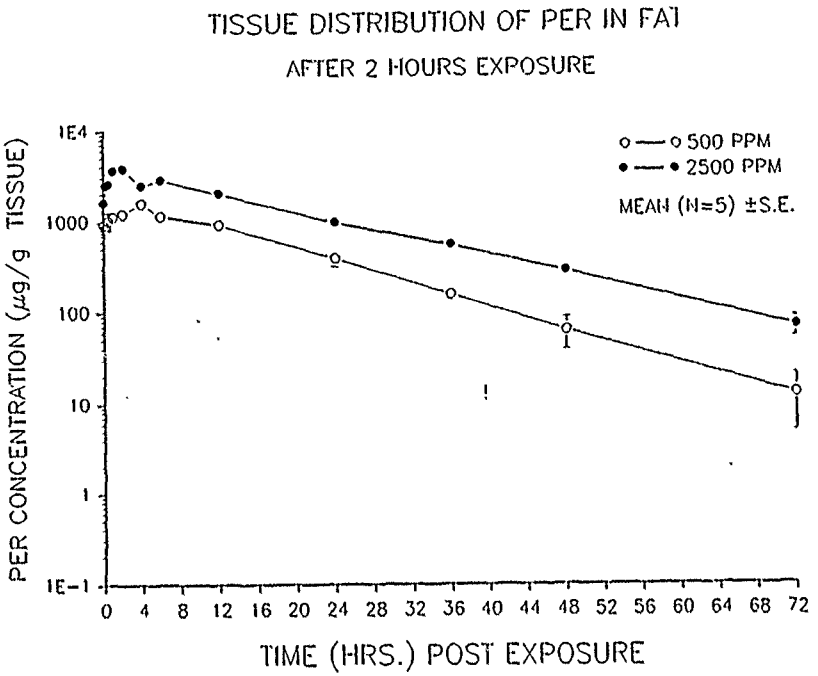

TISSUE DISTRIBUTION OF PER IN FAT
AFTER 2 HOURS EXPOSURE
o — o 500 PPM
• — • 2500 PPM
MEAN (N=5) ±S.E.
PER CONCENTRATION (μg/g TISSUE)
1E4
1000
100
10
1
1E-1
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
TIME (HRS.) POST EXPOSURE

Fig. K-3

TISSUE DISTRIBUTION OF PER IN HEART

AFTER 2 HOURS EXPOSURE

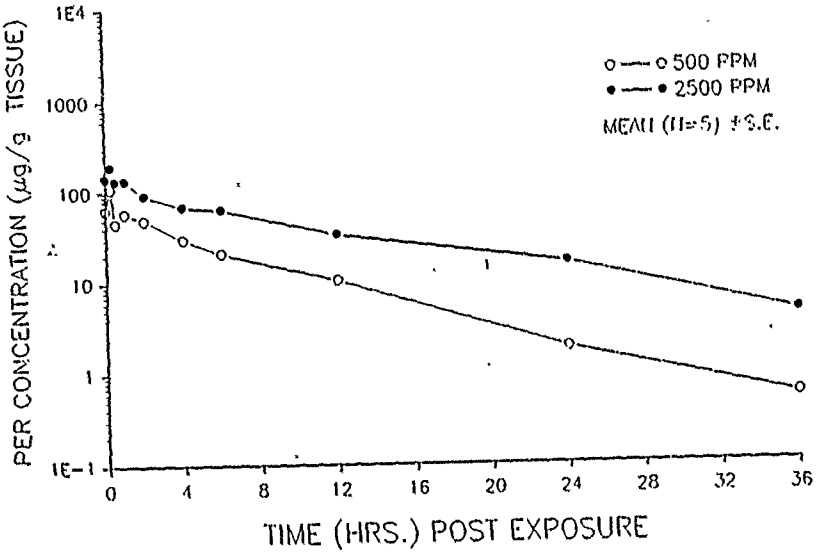


Fig. K-4

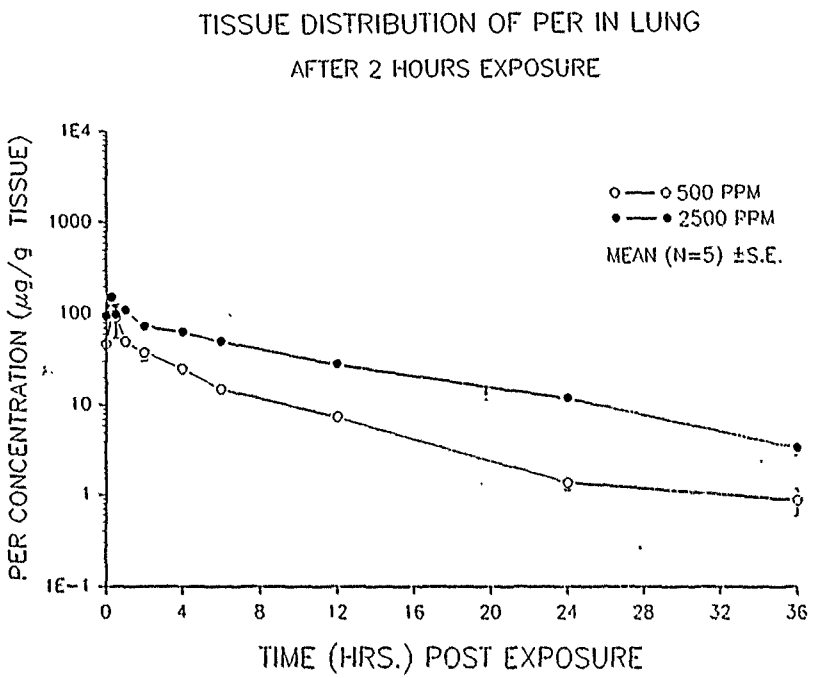

TISSUE DISTRIBUTION OF PER IN LUNG
AFTER 2 HOURS EXPOSURE
PER CONCENTRATION (μg/g TISSUE)
1E4
1000
100
10
1
1E-1
0 4 8 12 16 20 24 28 32 36
TIME (HRS.) POST EXPOSURE
o—o 500 PPM
•—• 2500 PPM
MEAN (N=5) ±S.E.

Fig. K-5

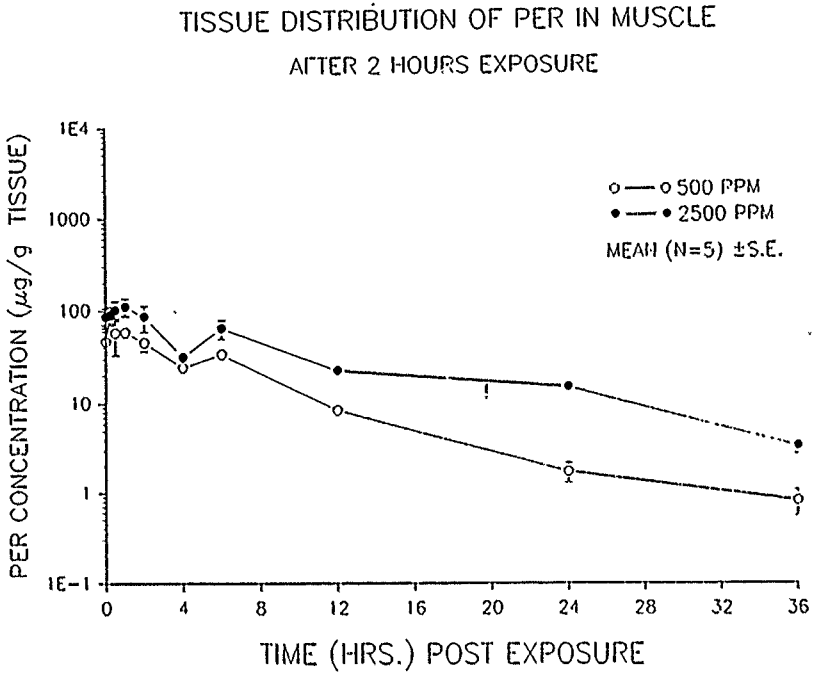

TISSUE DISTRIBUTION OF PER IN MUSCLE
AFTER 2 HOURS EXPOSURE
PER CONCENTRATION (μg/g TISSUE)
1E4
1000
100
10
1
1E-1
0 — o 500 PPM
• — • 2500 PPM
MEAN (N=5) ±S.E.
0
4
8
12
16
20
24
28
32
36
TIME (HRS.) POST EXPOSURE

Fig. K-6

TISSUE DISTRIBUTION OF PER IN BRAIN

AFTER 2 HOURS EXPOSURE

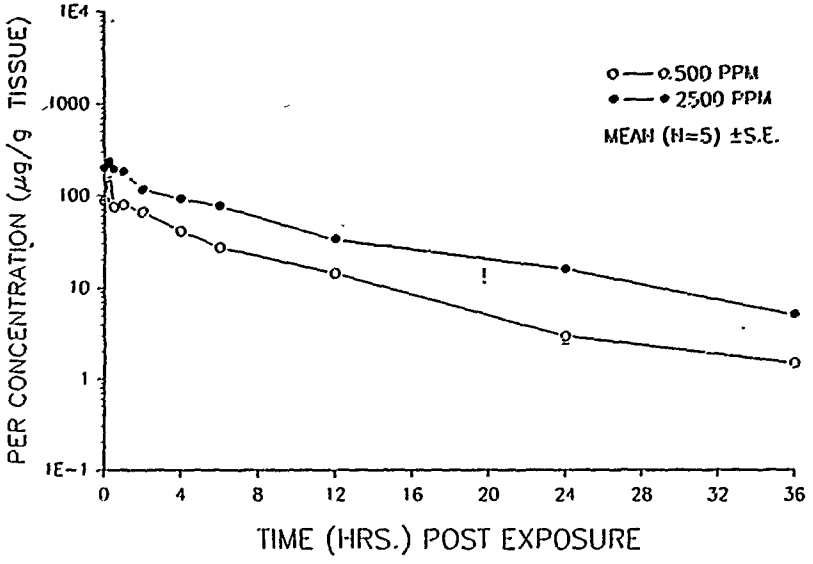


Fig. K-7

TISSUE DISTRIBUTION OF PER IN BLOOD

AFTER 2 HOURS EXPOSURE

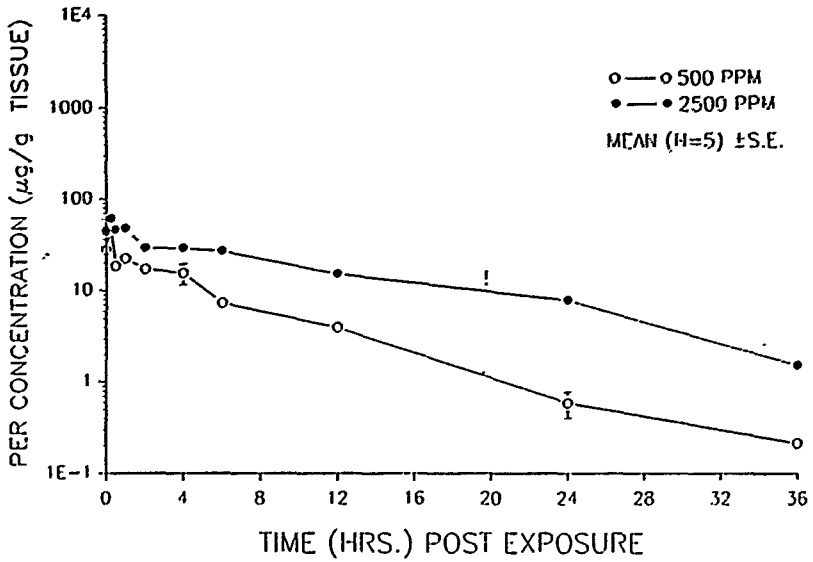


Fig. K-8

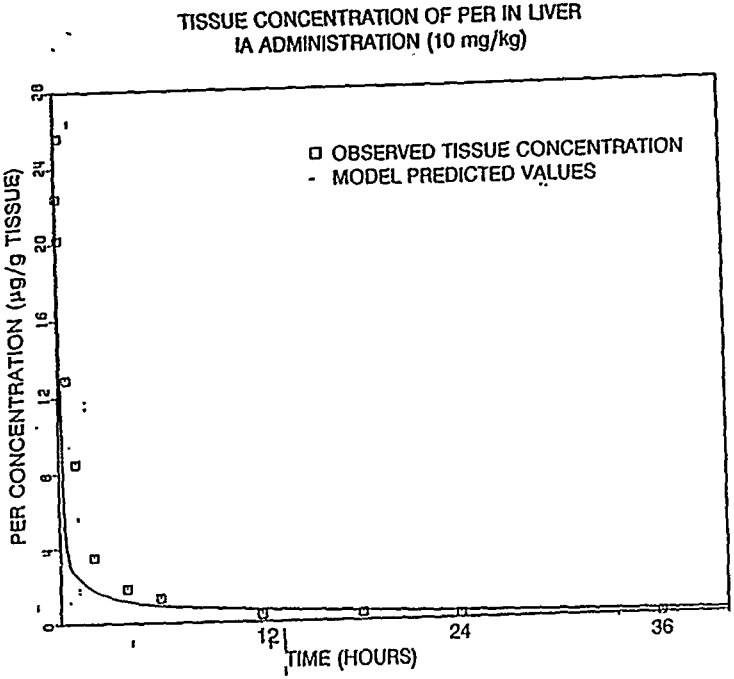

TISSUE CONCENTRATION OF PER IN LIVER
IA ADMINISTRATION (10 mg/kg)
OBSERVED TISSUE CONCENTRATION
MODEL PREDICTED VALUES
PER CONCENTRATION (µg/g TISSUE)
0
4
8
12
16
20
24
28
12
24
36
TIME (HOURS)

Fig. K-9

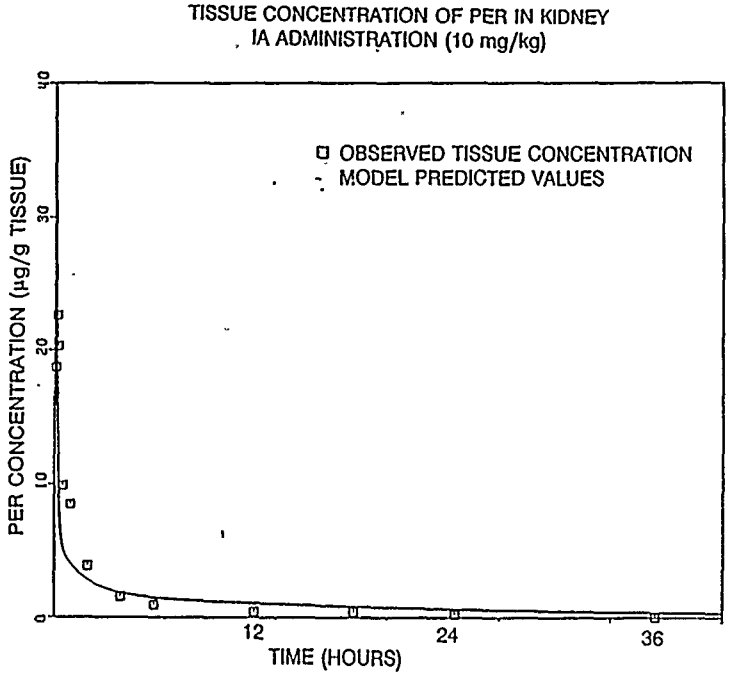

TISSUE CONCENTRATION OF PER IN KIDNEY
IA ADMINISTRATION (10 mg/kg)
OBSERVED TISSUE CONCENTRATION
MODEL PREDICTED VALUES
PER CONCENTRATION (µg/g TISSUE)
0
10
20
30
40
12
24
36
TIME (HOURS)

Fig. K-10

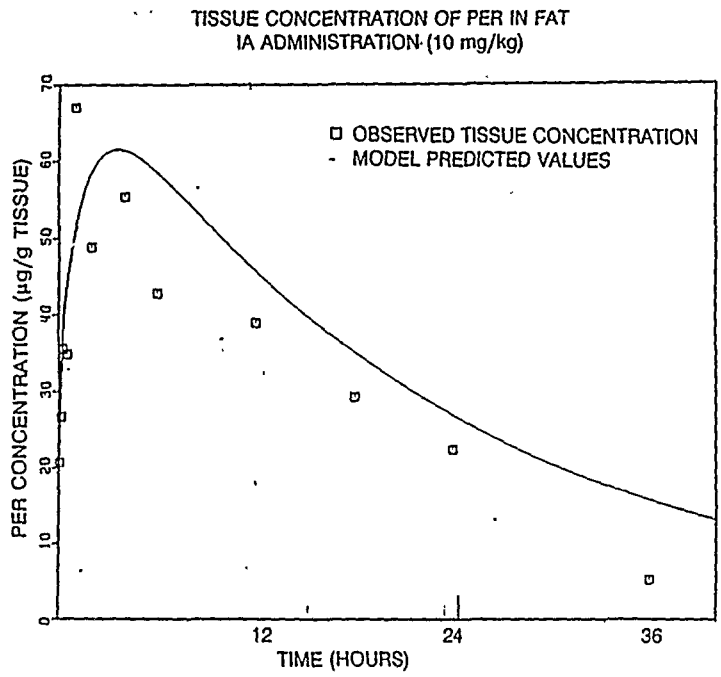

TISSUE CONCENTRATION OF PER IN FAT
IA ADMINISTRATION (10 mg/kg)
OBSERVED TISSUE CONCENTRATION
MODEL PREDICTED VALUES
PER CONCENTRATION (µg/g TISSUE)
0
10
20
30
40
50
60
70
12
24
36
TIME (HOURS)

Fig. K-11

TISSUE CONCENTRATION OF PER IN HEART
IA ADMINISTRATION (10 mg/kg)

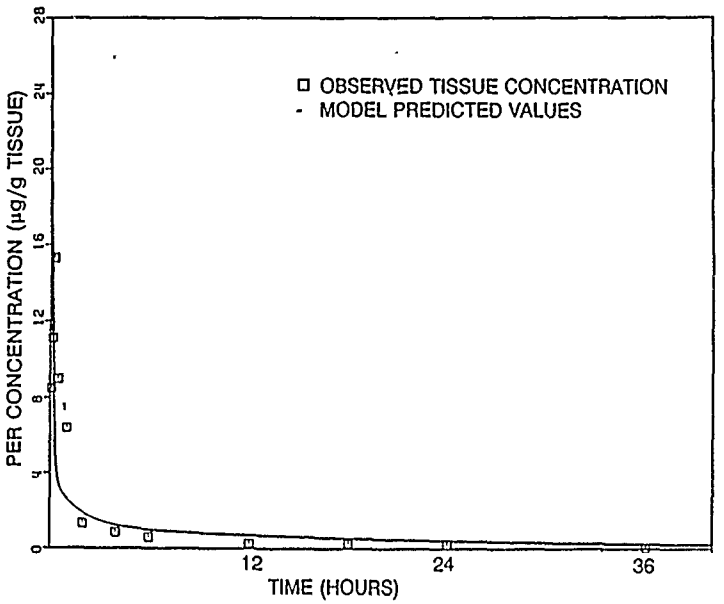


Fig. K-12

TISSUE CONCENTRATION OF PER IN LUNG
IA ADMINISTRATION (10 mg/kg)

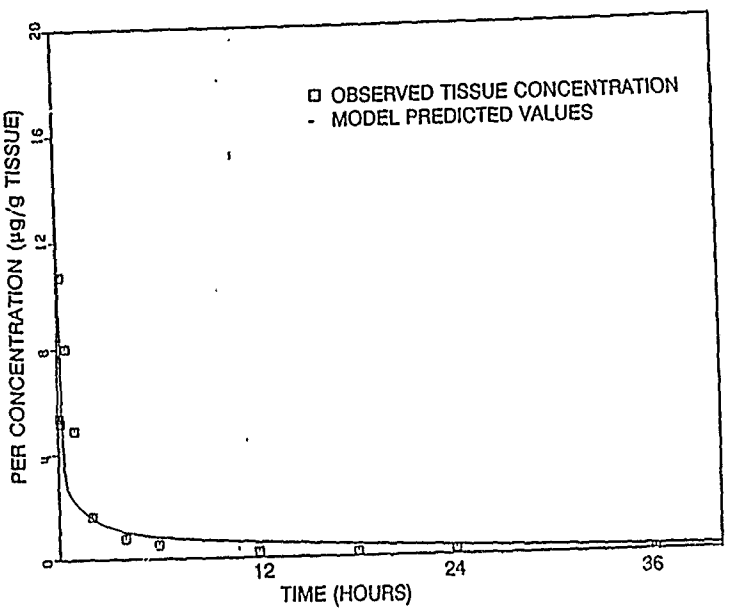


Fig. K-13

TISSUE CONCENTRATION OF PER IN MUSCLE
IA ADMINISTRATION (10 mg/kg)

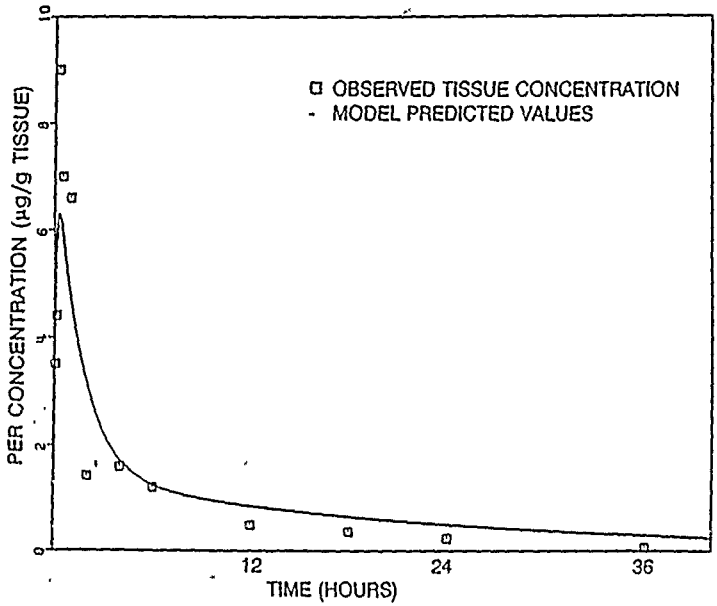


Fig. K-14

TISSUE CONCENTRATION OF PER IN BRAIN
IA ADMINISTRATION (10 mg/kg)

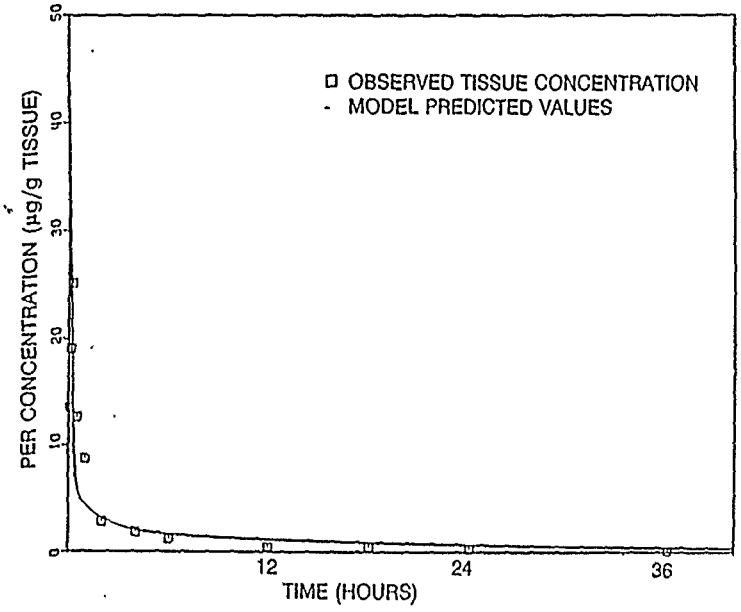


Fig. K-15

TISSUE CONCENTRATION OF PER IN BLOOD
IA ADMINISTRATION (10 mg/kg)

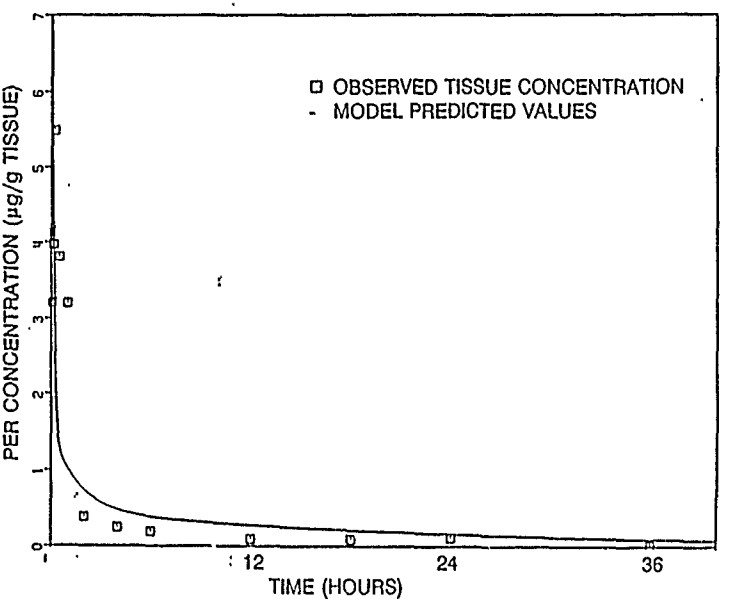


Fig. K-16

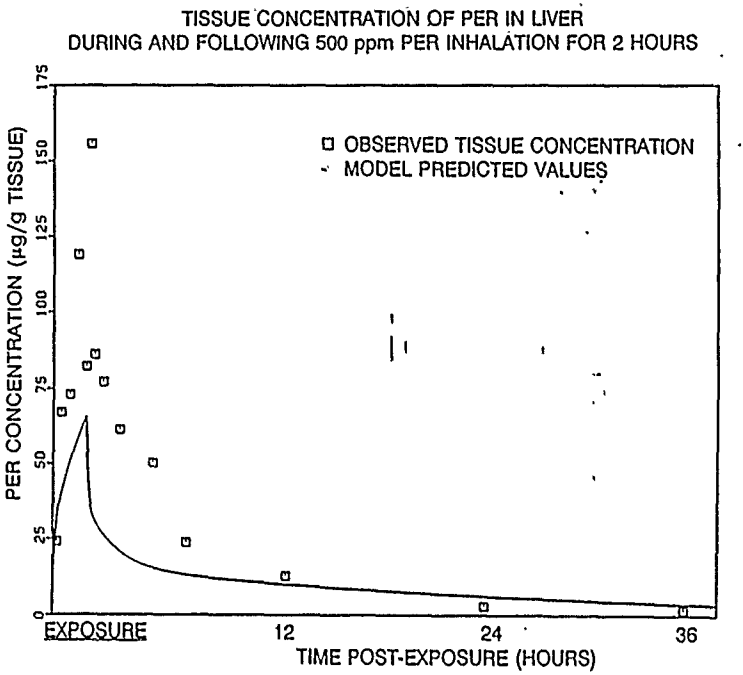

TISSUE CONCENTRATION OF PER IN LIVER
DURING AND FOLLOWING 500 ppm PER INHALATION FOR 2 HOURS
OBSERVED TISSUE CONCENTRATION
MODEL PREDICTED VALUES
PER CONCENTRATION (µg/g TISSUE)
0
25
50
75
100
125
150
175
EXPOSURE
12
24
36
TIME POST-EXPOSURE (HOURS)

Fig. K-17

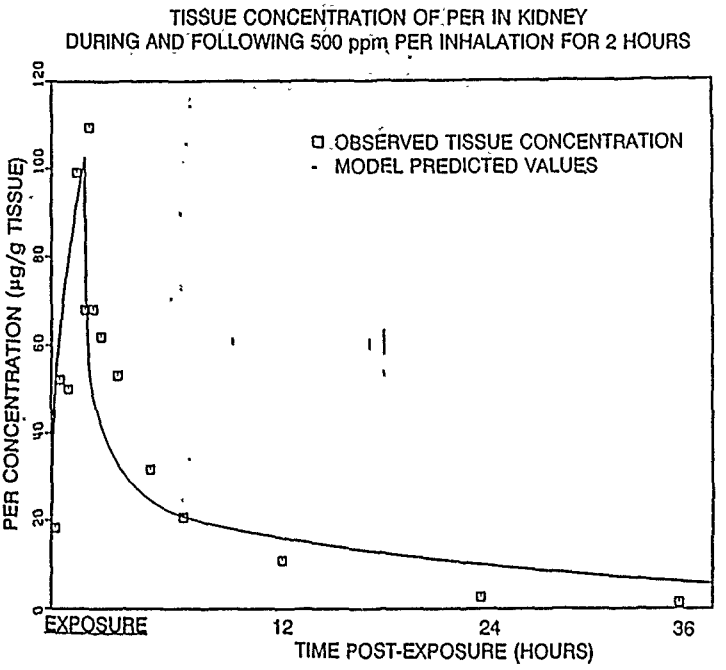

TISSUE CONCENTRATION OF PER IN KIDNEY
DURING AND FOLLOWING 500 ppm PER INHALATION FOR 2 HOURS
OBSERVED TISSUE CONCENTRATION
MODEL PREDICTED VALUES
PER CONCENTRATION (µg/g TISSUE)
0
20
40
60
80
100
120
EXPOSURE
12
24
36
TIME POST-EXPOSURE (HOURS)

Fig. K-18

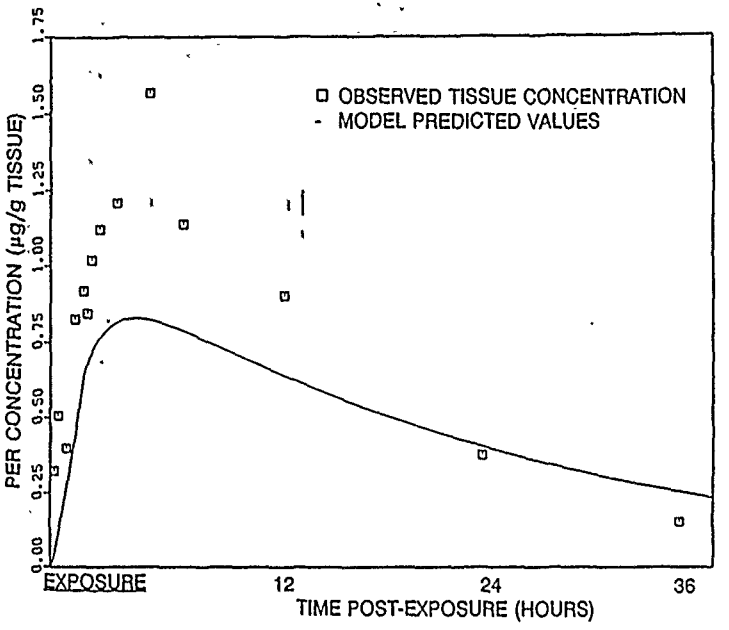

TISSUE CONCENTRATION OF PER IN FAT
DURING AND FOLLOWING 500 ppm PER INHALATION FOR 2 HOURS
OBSERVED TISSUE CONCENTRATION
MODEL PREDICTED VALUES
PER CONCENTRATION (µg/g TISSUE)
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
EXPOSURE
12
24
36
TIME POST-EXPOSURE (HOURS)

Fig. K-19

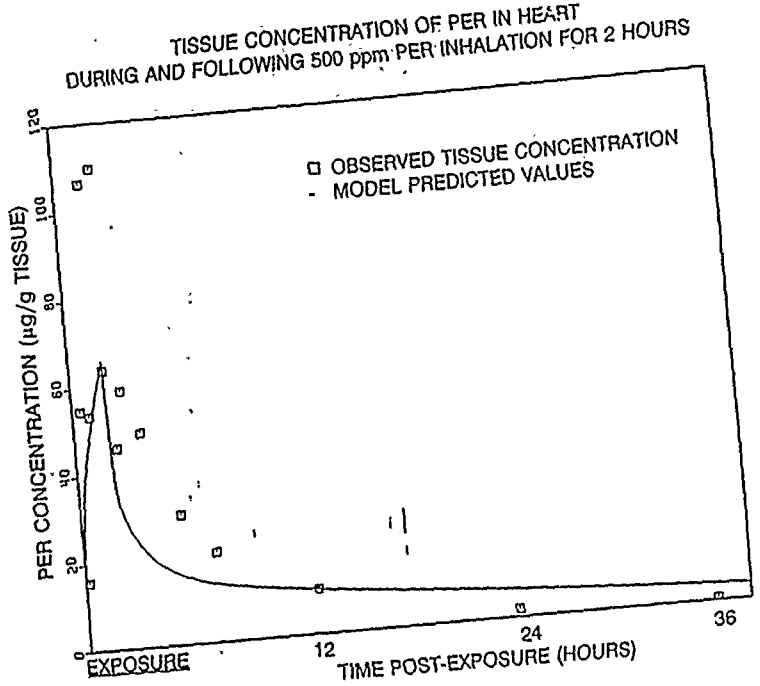

TISSUE CONCENTRATION OF PER IN HEART
DURING AND FOLLOWING 500 ppm PER INHALATION FOR 2 HOURS
OBSERVED TISSUE CONCENTRATION
MODEL PREDICTED VALUES
PER CONCENTRATION (µg/g TISSUE)
120
100
80
60
40
20
0
EXPOSURE
12
24
36
TIME POST-EXPOSURE (HOURS)

Fig. K-20

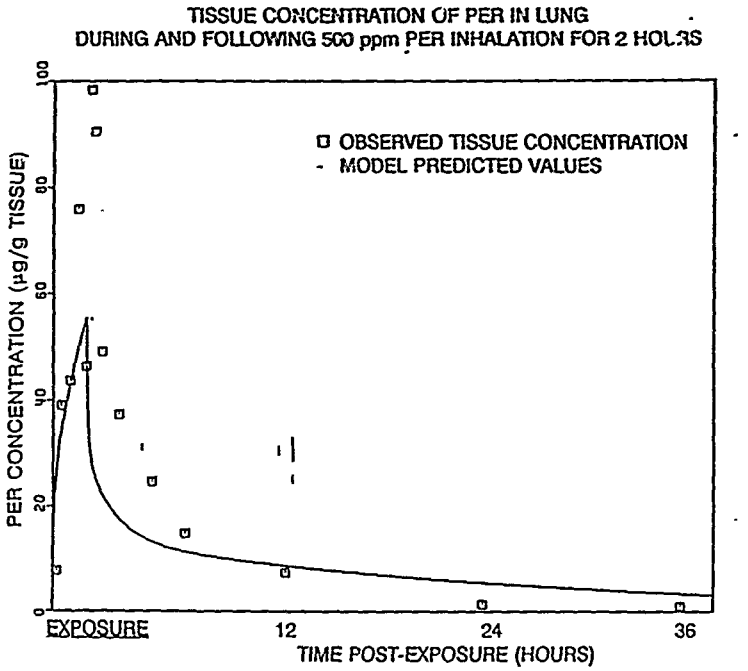

TISSUE CONCENTRATION OF PER IN LUNG
DURING AND FOLLOWING 500 ppm PER INHALATION FOR 2 HOURS
OBSERVED TISSUE CONCENTRATION
MODEL PREDICTED VALUES
PER CONCENTRATION (µg/g TISSUE)
0
20
40
60
80
100
EXPOSURE
12
24
36
TIME POST-EXPOSURE (HOURS)

Fig. K-21

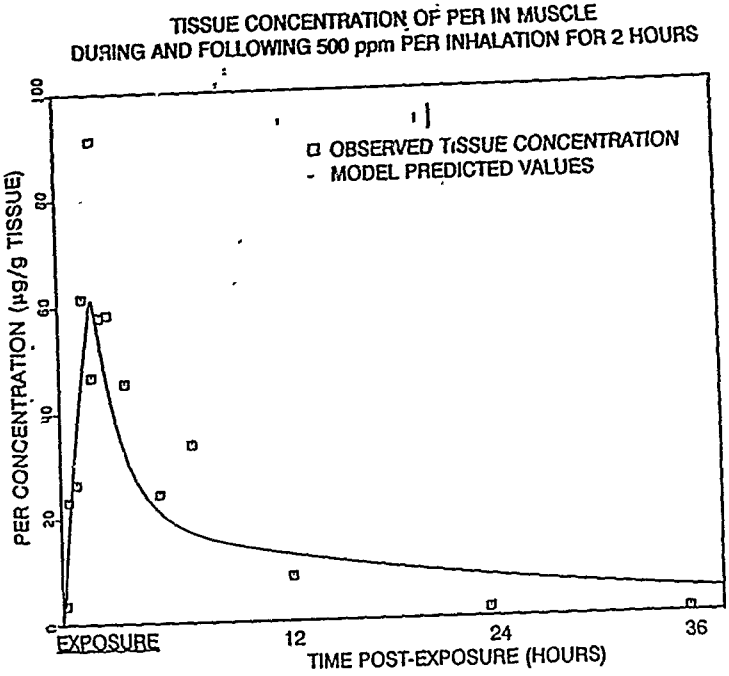

TISSUE CONCENTRATION OF PER IN MUSCLE
DURING AND FOLLOWING 500 ppm PER INHALATION FOR 2 HOURS
OBSERVED TISSUE CONCENTRATION
MODEL PREDICTED VALUES
PER CONCENTRATION (µg/g TISSUE)
100
80
60
40
20
0
EXPOSURE
12
24
36
TIME POST-EXPOSURE (HOURS)

Fig. K-22

TISSUE CONCENTRATION OF PER IN BRAIN
DURING AND FOLLOWING 500 ppm PER INHALATION FOR 2 HOURS

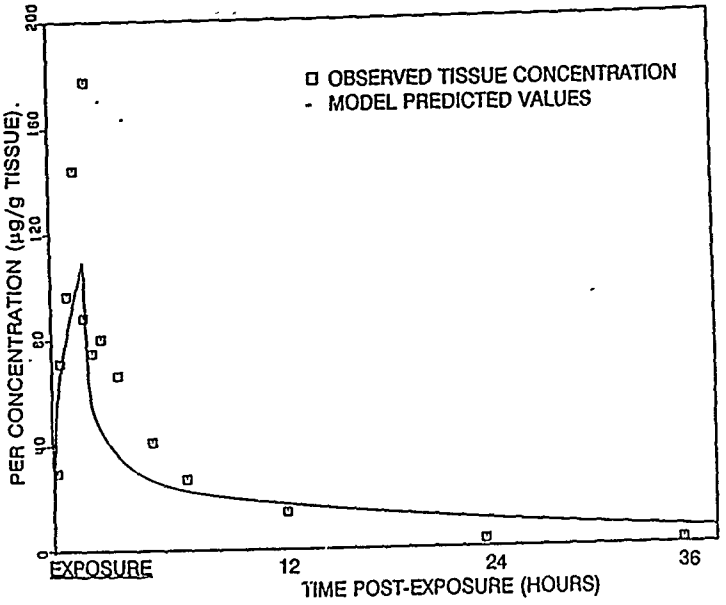


Fig. K-23

TISSUE CONCENTRATION OF PER IN BLOOD
DURING AND FOLLOWING 500 ppm PER INHALATION FOR 2 HOURS

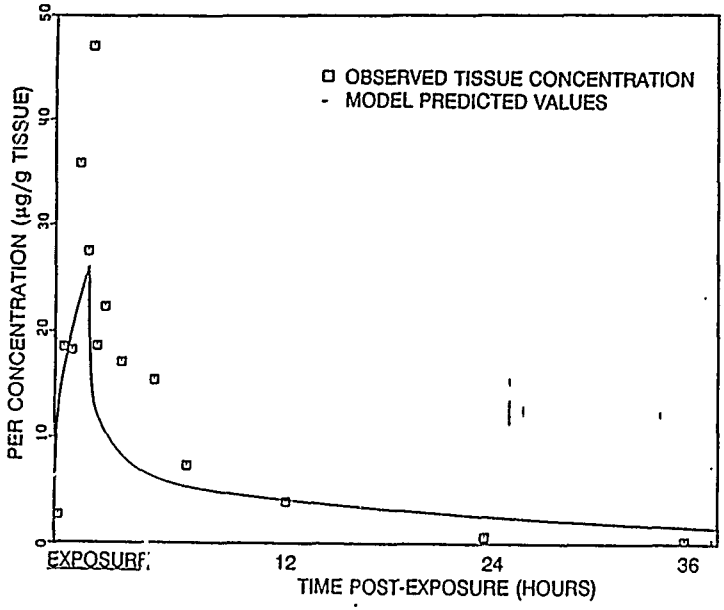


Fig. K-24

Simulation of PER tissue concentrations

The physiologically-based pharmacokinetic model for PER is taken from [1] with two major differences:

a./ the elimination from the liver is not linear but Michealis-Menten type,

b./ the richly perfused compartment is divided into four compartments: heart, kidney, brain and richly perfused.

The subscripts in the model and in the other part of the text are as follows:

H	-	heart	BR	-	brain
K	-	kidney	M	-	muscle
R	-	richly perfused	Li	-	liver
F	-	fat	L	-	lung
A	-	alveolar space	V	-	venous blood
BL	-	arterial blood	T	-	total (blood)

Constants of the model for 339 g rat

Blood flows (ml/min)

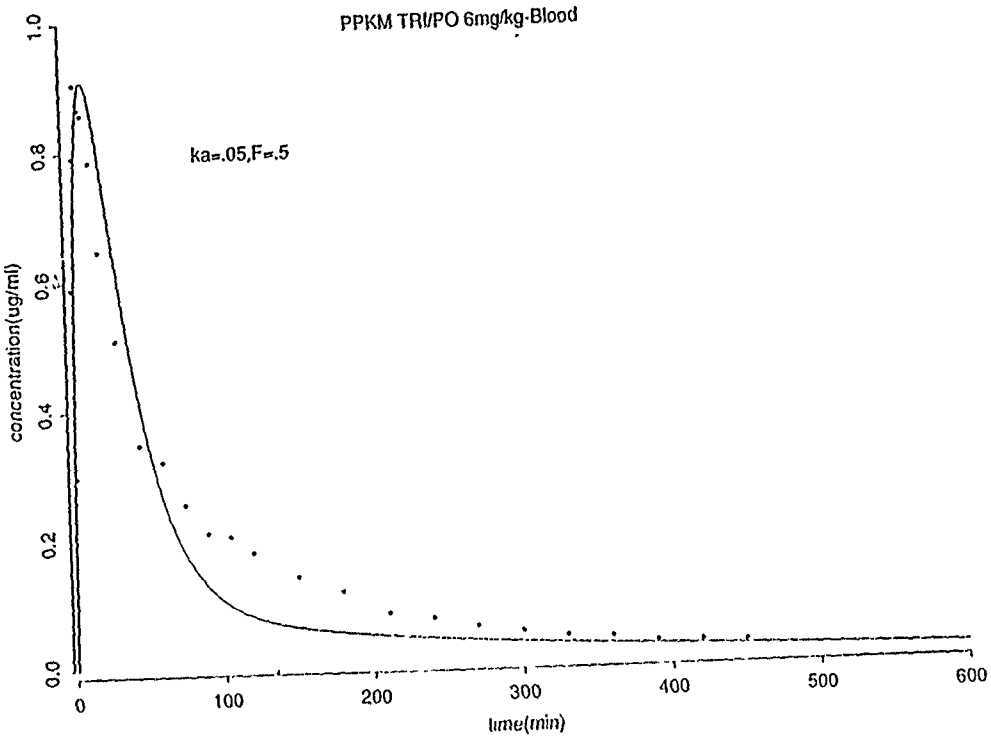
Q_T	=	104.4	Q_H	=	3.03
Q_{BR}	=	1.25	Q_K	=	18.27
Q_M	=	15.66	Q_{Li}	=	26.73
Q_F	=	9.4	Q_R	=	30.06 (different from [1] to satisfy Q_T)

Tissue volumes (ml)

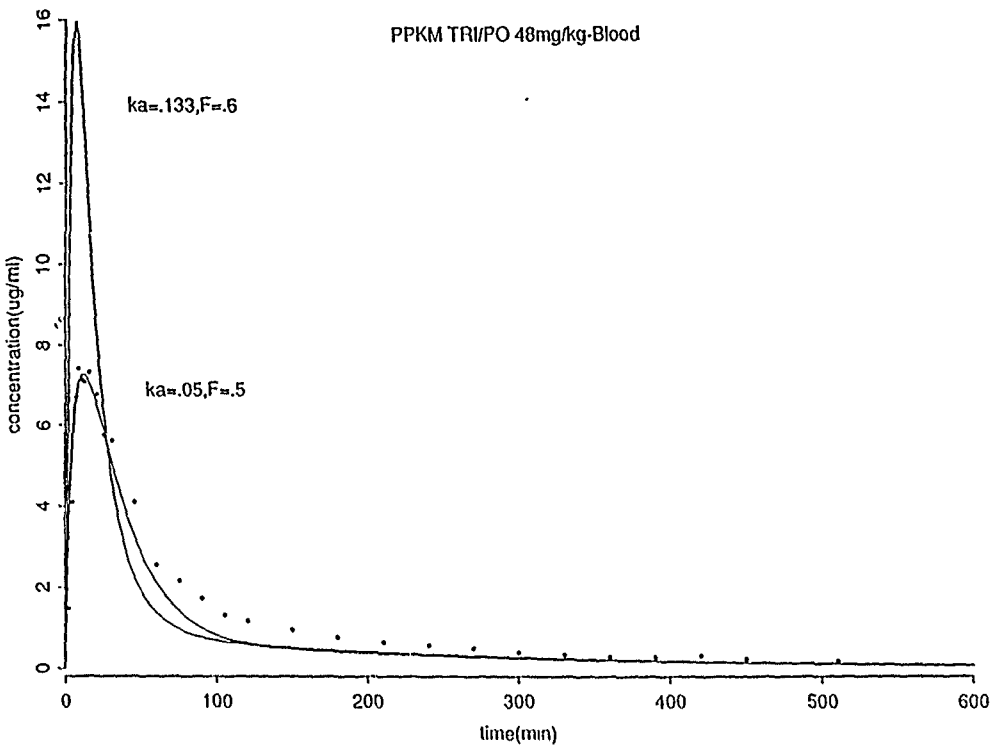
V_{BL}	=	25.32	V_H	=	1.7
V_{BR}	=	0.98	V_K	=	3.19
V_M	=	247.13	V_R	=	11.09
V_{Li}	=	13.56	V_F	=	30.17
V_A	=	1.9			

Alveolar ventillation rate	VR_A	=	140 ml/min
Lung-alveolar mass transfer coeff.	h	=	500 ml/min
Michaelis-Menten constants	k_m	=	2.9378 ug/min
	V_m	=	5.86 ug/ml
Dose	D	=	3390 ug
Richly perf.:Blood part. coeff.	R_R	=	3.72 (from [1])
Lung:Air partition coefficient	R_A	=	70.3

Table K-3



Observed (•) and model-predicted (-) TRI concentrations in the blood of rats that have received a single oral bolus administration of 6 mg/kg TRI in emulphor. In the physiologically-based pharmacokinetic model used for the simulation of TRI ingestion, a bioavailability of 0.5 and a Ka of 0.05 were employed. Each observed value is the mean for 8 rats.



Observed (•) and model-predicted (-) TRI concentrations in the blood of rats that have received a single oral bolus administration of 48 mg/kg TRI in emulphor. In the physiologically-based pharmacokinetic model used for the simulation of TRI ingestion, a bioavailability of 0.5 and a Ka of 0.05 were employed. Each observed value is the mean for 6 rats.

Fig. K-26

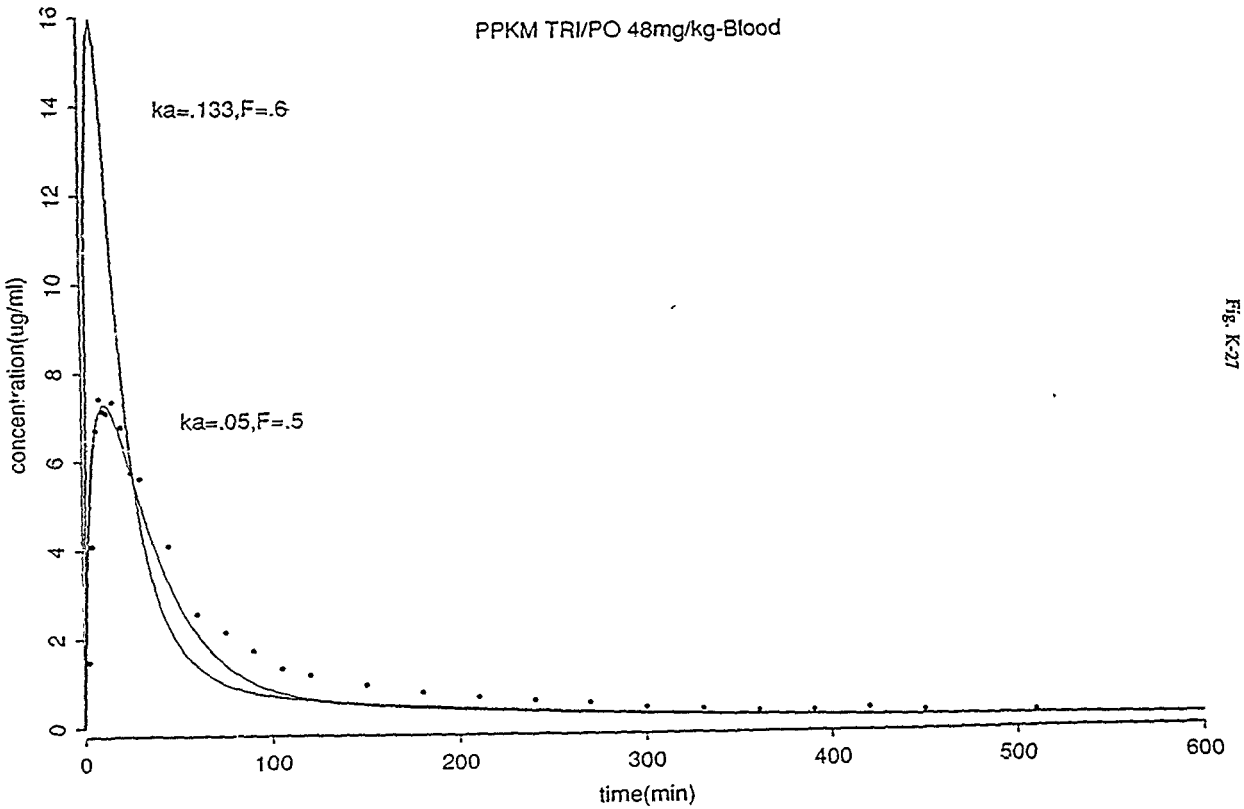
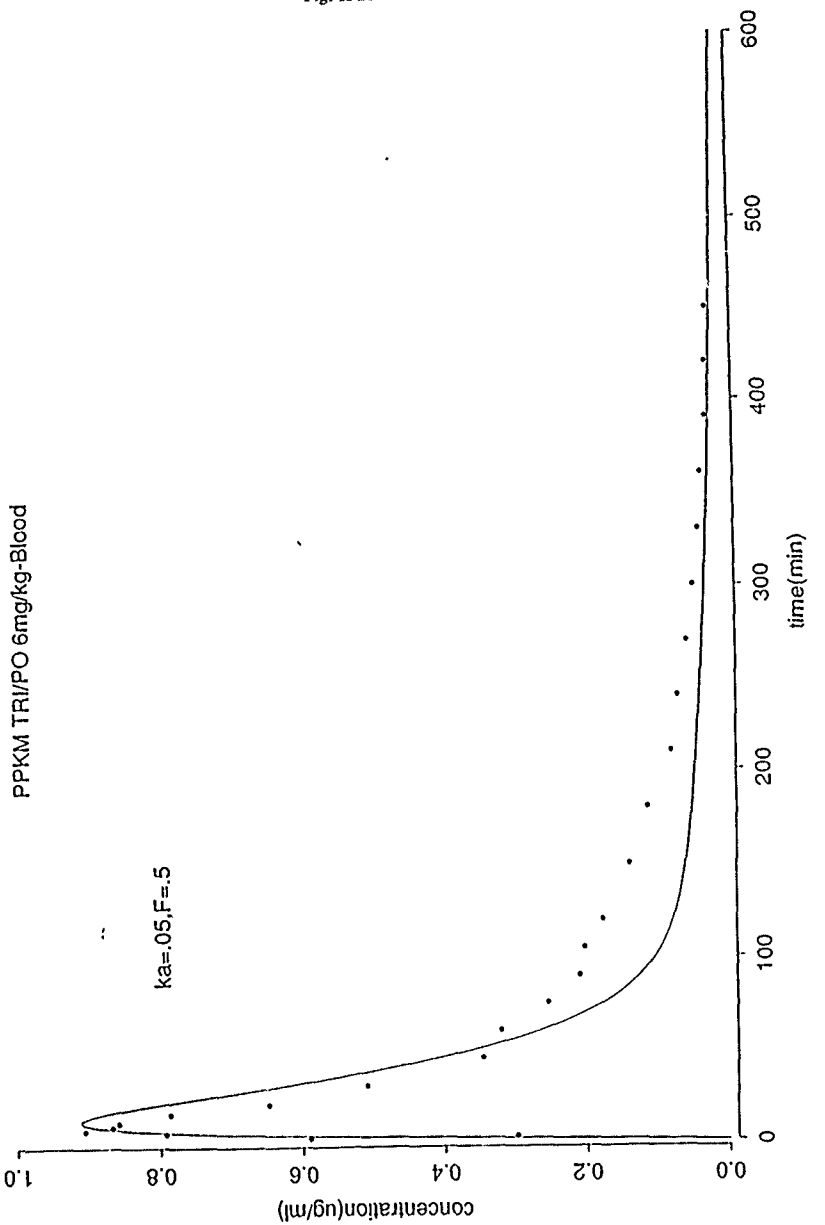

PPKM TRI/PO 48mg/kg-Blood
ka=.133,F=.6
ka=.05,F=.5
concentration(ug/ml)
time(min)
0
2
4
6
8
10
12
14
16
0
100
200
300
400
500
600

Fig. K-27

Fig. K-28


PPKM TRI/PO 6mg/kg-Blood
ka=.05,F=.5
concentration(ug/ml)
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
time(min)

APPENDIX L

MEASUREMENTS OF THE NEUROBEHAVIORAL TOXICITY OF HALOCARBONS

FIGURE 1

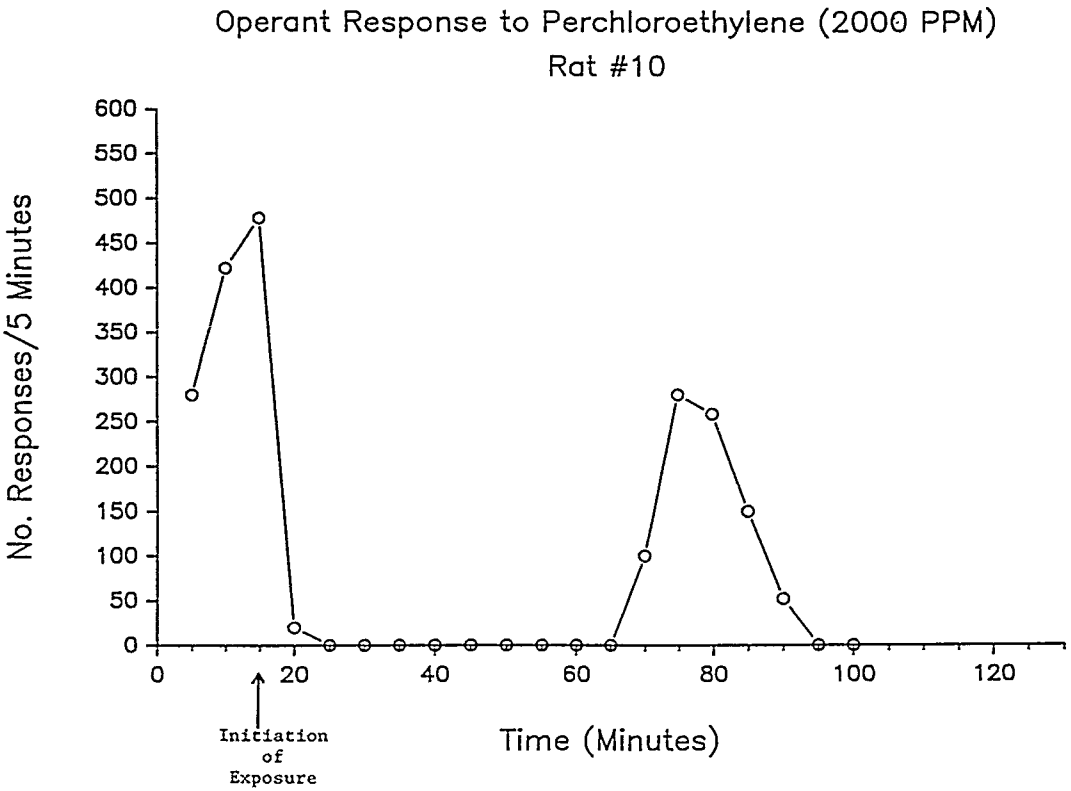


Fig. L-1

FIGURE 2

Operant Response to Perchloroethylene (2000 PPM)
RAT #11

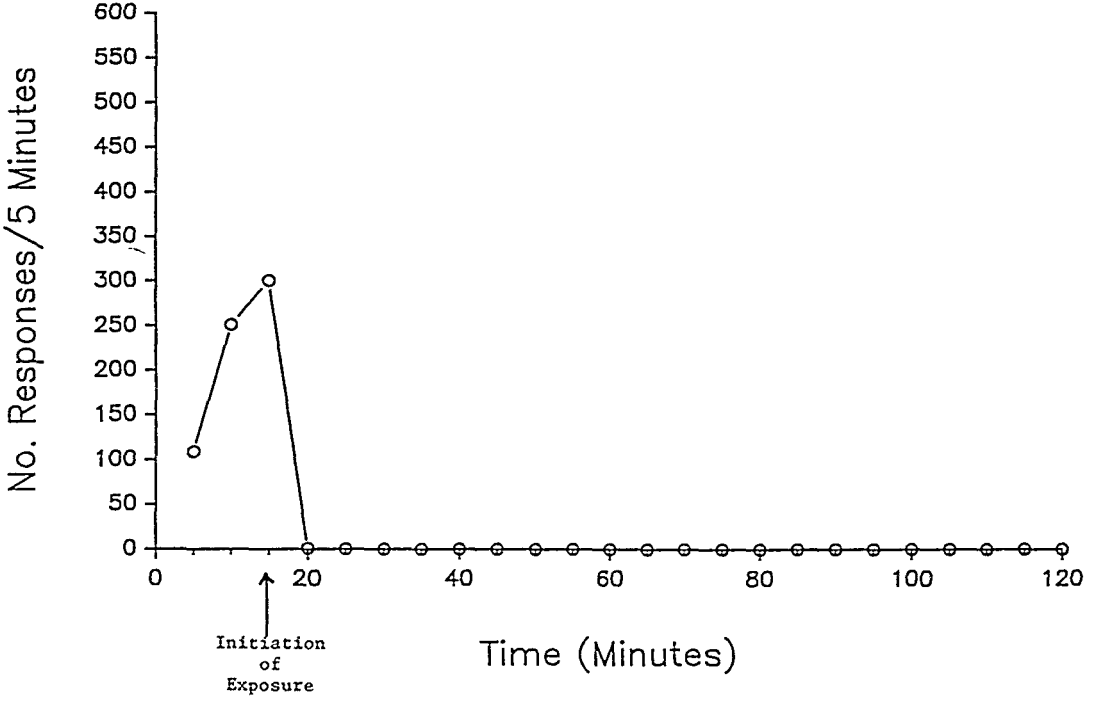


Fig. L-2

FIGURE 3

Operant Response to Perchloroethylene (500 PPM)

Rat #8

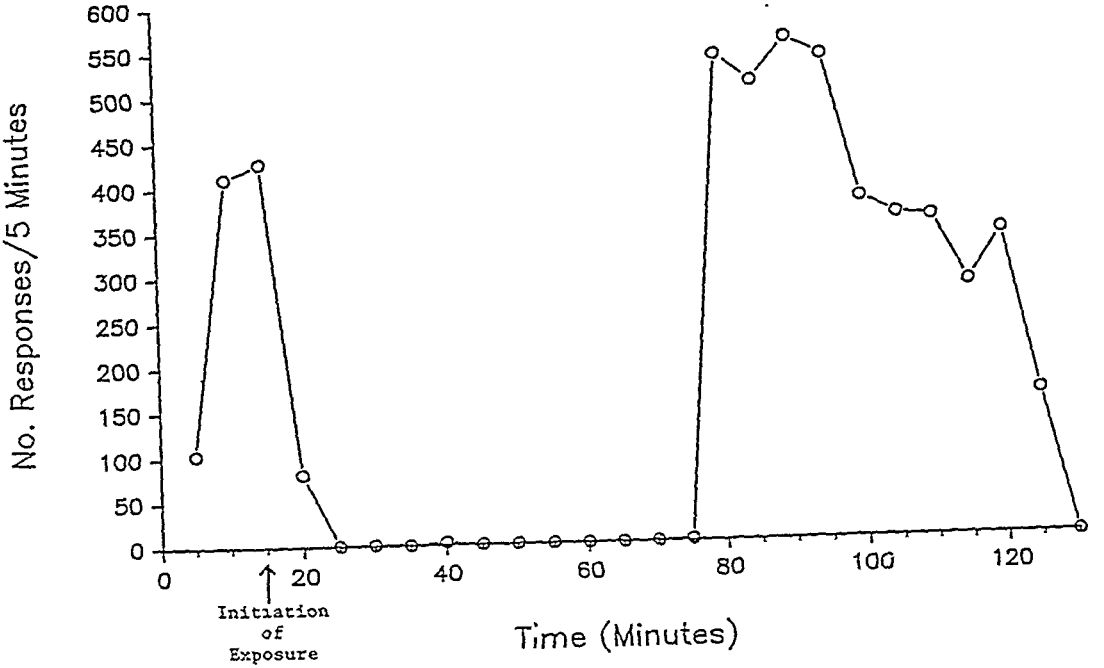


Fig. L-3

FIGURE 4

Operant Response to Perchloroethylene-
Rat #8 vs. Rat #10

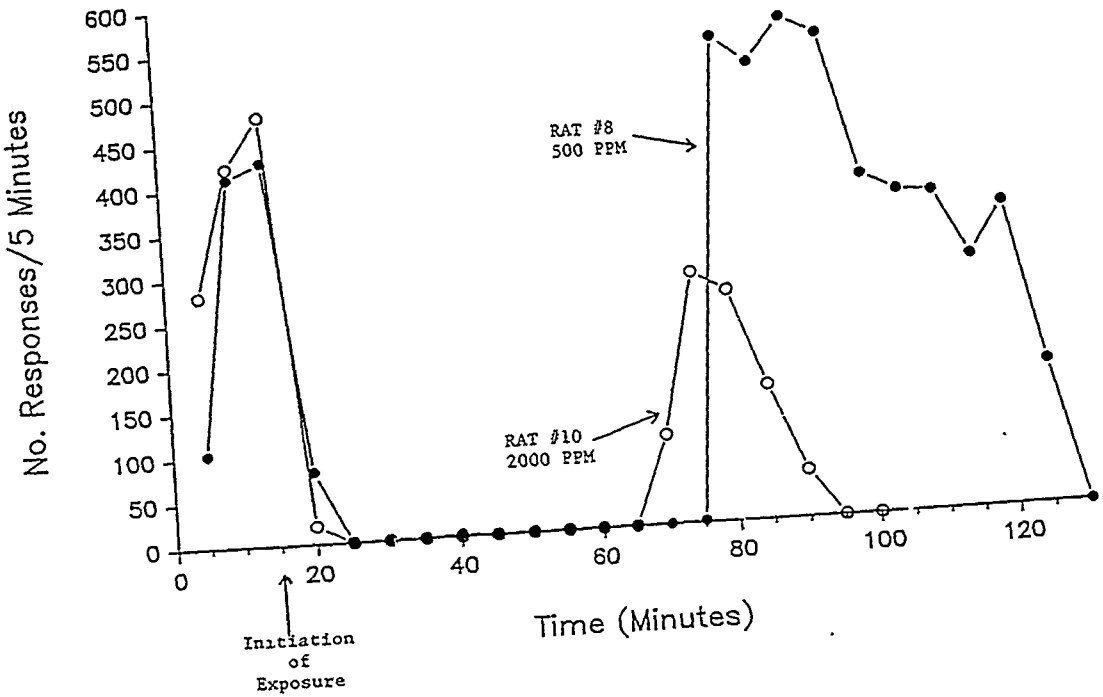


Fig. I-4

APPENDIX H

Cumulative and Chronological List of Research Papers and Abstracts

1. Dallas, C.E., Ramanathan, R., Gallo, J.M., Dallas, C.E., and Bruckner, J.V. "Comparative pharmacokinetics of inhaled and ingested 1,1-dichloroethylene (DCE) in rats." Toxicologist 8: 139 (1988).

2. Ramanathan, R., Muralidhara, S., Gallo, J.M., Dallas, C.E., and Bruckner, J.V. "Pharmacokinetics of volatile halocarbons: Comparison of single oral bolus versus infusion of trichloroethylene (TCE)." Toxicologist 8: 94 (1988).

3. Muralidhara, S., Ramanathan, R., Gallo, J.M., Dallas, C.E., and Bruckner, J.V. "Pharmacokinetics of volatile halocarbons: Comparison of single oral bolus versus gastric infusion of 1,1,1-trichloroethane (TRI)." Toxicologist 8: 95 (1988).

4. Gallo, J.M., Dallas, C.E., and Bruckner, J.V. "Physiological pharmacokinetic models for 1,1,1-trichloroethane (TRI) and 1,1,1-trichloroethane (TCE) in rats following inhalation and oral exposures." Toxicologist 9: 230 (1989).

5. Ramanathan, R., Muralidhara, S., Dallas, C.E., Gallo, J.M., and Bruckner, J.V. "Influence of the pattern of ingestion on the pharmacokinetics of perchloroethylene (PER) in rats." Toxicologist 9: 78 (1989).

6. Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., Manning, R.O., and Bruckner, J.V. "Direct measurements of perchloroethylene in the blood and exhaled breath of rats during and following inhalation exposure." Toxicologist 9: 78 (1989).

7. Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "The uptake and elimination of 1,1,1-trichloroethane (TRI) during and following inhalation exposures in rats. Toxicology and Applied Pharmacology 98: 385-397 (1989).

8. Chen, X.M., Dallas, C.E., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "Tissue disposition of ingested perchloroethylene (PER) in rats." Toxicologist 10: 235 (1990).

9. Dallas, C.E., Muralidhara, S., Chen, X.M., Stevens, E.K., Martin, J.E., and Irvin, T.R. "Maternal and embryonic disposition of oral trichloroethylene (TCE)." Toxicologist 10: 208 (1990).

10. Dallas, C.E., Chen, X.M., Muralidhara, S., Tackett, R.L., Bruckner, J.V., and Gallo, J.M. "Interspecies comparisons of the toxicokinetics and bioavailability of ingested tetrachloroethane." Global Environmental Issues: Challenge for the 90s: 176 (1990).

11. Chen, X.M., Dallas, C.E., Muralidhara, S., Tackett, R.L., Bruckner, J.V., and Gallo, J.M. "Interspecies comparisons of perchloroethylene pharmacokinetics following oral and intraarterial administration. Toxicologist 11: 351 (1991).

12. Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats." In Press, Toxicology and Applied Pharmacology, (1991).

13. Dallas, C.E., Gallo, J.M., Chen, X.M., Muralidhara, S., O'Barr, K., and Bruckner, J.V. "Physiologically-based model parameter estimation from perchloroethylene tissue pharmacokinetics." Toxicologist 11: 33 (1991).

14. Chen, X.M., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. "Determination of volatile short-chain aliphatic halocarbons in animal tissues." Now being submitted to the Journal of Environmental Pathology, Toxicology, and Oncology (1991).

15. Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "Comparative pharmacokinetics of inhaled and ingested 1,1-dichloroethylene in rats." (To be submitted to Toxicology and Applied Pharmacology, 1991).